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NEURO-ONCOLOGY

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CELL BIOLOGY AND SIGNALING

CB-001. IKAROS EXPRESSION INHIBITS GBM TUMORIGENESIS AND CORRELATES WITH A POSITIVE PROGNOSIS

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Ikaros, a DNA-binding protein also known as Ikaros family zinc finger protein 1 (IKZF1), was initially shown to function critically in hematopoietic differentiation. Deletion or mutation of Ikaros has been associated with lymphoblastic, as well as acute and chronic myelogenous leukemias demonstrating that Ikaros can act as a tumor suppressor. Ikaros is predominantly expressed in a variety of tissue including the brain, suggesting that Ikaros may be involved in the physiological functions of glial growth. Other recent studies have shown that Ikaros can regulate the transition of neural progenitor cells to postmitotic neurons which eventually differentiate into glial cells. Deregulation of glial development and/or growth has been shown to initiate glioblastoma (GBM) growth suggesting that Ikaros may be involved in GBM tumorigenesis. The goal of this study was to determine if Ikaros plays an important role in glial tumorigenesis. Analysis using the TCGA and Rembrandt databases demonstrated that decreased expression of Ikaros leads to worse prognosis in GBM patients ($P < 0.01$). In addition, the Ikaros promoter was found to be hypermethylated (85% of samples) in GBM samples suggesting that inhibition of Ikaros may contribute to glial tumorigenesis. To determine if Ikaros is involved in glial tumorigenesis we generated stable GBM cell lines that were transduced with HA-Ikaros. Enhanced expression of Ikaros resulted in a 3- to 4-fold reduction in proliferation and a 2- to 3-fold reduction in colony formation. In addition, exogenous expression of Ikaros inhibited the migration of GBM cells suggesting that Ikaros may also be involved in the invasiveness of glial cancers. Together our data suggests that Ikaros may play an important role in suppressing glial tumor formation and may be a novel biomarker for GBMs.

CB-002. MUTATIONS IN THE *TERT* PROMOTER ARE HIGHLY RECURRENT AND UPREGULATE *TERT* EXPRESSION IN GLIOMAS

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Telomere lengthening is mandatory for infinite proliferation of cancer cells, and the majority of cancers including gliomas achieve this by activating telomerase. However, the pathogenesis of telomerase upregulation has been unknown. To investigate the mechanism of telomerase activation in gliomas, we analyzed mutations in the promoter of the telomerase reverse transcriptase (*TERT*) in 546 glioma samples. High incidence of mutations was observed at two hot spots, C228T and C250T, in all subtypes of adult gliomas (298/546, 55%). The frequency of mutations was higher in primary glioblastomas (179/256, 70%) and pure oligodendrogliomas (48/65, 74%), compared with those in diffuse astrocytomas (10/52, 19%) or anaplastic astrocytomas (20/79, 25%). The expression levels of *TERT* mRNA in tumors carrying those mutations were significantly higher than that of the wild type tumors (mean >6 times), indicating that the mutated promoter leads to upregulation of *TERT*. *TERT* promoter mutations were concomitant with total 1p19q loss in all but one cases (78/80 cases, 98%), however rare in tumors with *IDH1/2* mutations but not with total 1p19q loss (8/100, 8%). Most *TERT* promoter-mutated glioblastomas had wild-type *IDH1/2* and no total 1p19q loss (156/160, 98%). In addition, most *EGFR* amplifications (68/74, 93%) were associated with *TERT* promoter mutations. Thus, we show that *TERT* promoter mutations play an important role in the pathogenesis of gliomas, particularly in oligodendrogliomas and glioblastomas. As telomerase activity is known to correlate with *TERT* expression, our data indicate that the *TERT* promoter mutation is one of the major mechanisms of telomerase activation in gliomas. The distinct patterns of *TERT* promoter mutations in relation to *IDH1/2* or 1p19q copy number status suggest that *TERT* mutations are involved in the development of oligodendroglial and glioblastomas through different mechanisms.

CB-003. ACTIVATION OF THE Wnt/β-CATENIN PATHWAY INHIBITS GLIOBLASTOMA STEM CELLS SELF-RENEWAL

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Cancer stem cells (CSCs) constitute a subpopulation of cells within tumors that promote tumor growth and recurrence. Resistance to current cancer treatments suggests that these therapies, while killing the majority of tumor cells, may finally fail because they are not able to eliminate CSCs, which survive to regenerate new tumors. We found that Frizzled is the most expressed G protein-coupled receptor (GPCR) family in glioblastoma (GBM) stem cells (GSCs) and set out, therefore, to investigate the function of the canonical Wnt pathway in these cells. We were able to show that activation of the Wnt/β-catenin pathway inhibits GSC self-renewal and the expression of undifferentiation markers. Moreover, the alkylating agent temozolomide (TMZ), currently used as a standard protocol for GBM treatment, induces p53 expression, which in turn stimulates Dkk1 (an inhibitor of the Wnt pathway) production in GSCs. Therefore, treatment with TMZ induces the undifferentiated status and the self-renewal of GSCs, which might explain the recurrence of GBM and the resistance of GSCs to chemotherapy treatments.

CB-004. TUMOR MIGRATION OF HUMAN GLIOBLASTOMA IS MODULATED BY THE MOLECULE CD90 (Thy-1)

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The molecule CD90 is a N-glycosylated, glycophosphatidylinositol anchored cell surface protein, originally described on thymocytes. CD90 has been considered as a surrogate marker for a variety of stem cells and has recently been reported on glioblastoma stem cells. CD90 is also expressed on T lymphocytes, endothelial cells, fibroblasts and neurons. The function of CD90 is not fully elucidated. CD90 has been involved in cell-cell and cell-matrix interactions, in neurite outgrowth, T cell activation and apoptosis. In this study, we confirmed the expression of CD90 on human glioblastoma stem-like cells from serum-free neurosphere cultures. We also observed RNA and protein CD90 expression on primary cell lines from FSC-containing culture (adherent cell lines) and on freshly prepared glioblastoma specimen. In order to study the function of CD90 on glioblastoma cells, we used a silencing strategy to decrease the expression of CD90 on the immortalized U251 cell line. We then compared the viability, the tumor growth and the migration property of the wild-type CD90+ U251 cells and CD90 down-regulated U251 clones. The decrease of CD90 expression did not affect the viability and the tumor growth of U251 cells. In contrast, down-regulation of CD90 mediated the decreased ability of tumor cell migration using both scratch wound healing and boyden chamber migration assays. Experiments are currently on going to test the effect of CD90 expression on tumorigenicity in mice models. In total, this study might lead to better understand the role of CD90 on the pathology in particular in term of tumor migration/invasion of human glioblastoma.

CB-005. ALK ALTERATIONS IN A SERIES OF 62 GLIOBLASTOMA CASES: IS THERE A THERAPEUTIC ROLE FOR ALK INHIBITORS MOLECULES?

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BACKGROUND: In the last years structural and point mutations involving ALK have been described in a number of different human cancer types. These alterations have constituted the basis for the potential use of ALK inhibitor molecules as targeted therapy in some non-small cell lung cancer, lymphoma

and neuroblastoma cases. MATERIAL AND METHODS: Using tissue microarrays, we have immunohistochemically studied ALK protein expression with three different commercial antibodies, as well as ALK copy number alterations by FISH using a dual-color break-apart probe (Vysis, Abbott), in a series of 62 glioblastoma (GB) cases. RESULTS: None of the 62 GB cases showed ALK expression with any of the three antibodies employed. A total of 33 out of the 52 GB cases that were evaluable by FISH showed copy number gain (defined as more than 2 copies in at least 10% of tumor cells). In one of these 33 cases more than 6 ALK copies (probably indicating gene amplification) were observed, always in multinucleated tumor cells. The remaining 19 cases showed 2 ALK copies (18 cases) or a solitary ALK copy (1 case). None of the cases demonstrated gene rearrangement. CONCLUSIONS: Our results are not concordant with data reported by other authors who, using the same methodology employed here, have found ALK protein overexpression and gene amplification in GB. Although point mutations should also be studied in GB patients, our preliminary results do not support the use of ALK inhibitors as a potential therapeutic option for GB patients.

CB-006. TARGETING THE PROTEIN KINASE CK2 SUPPRESSES PRO-SURVIVAL SIGNALING PATHWAYS AND GROWTH OF GLIOBLASTOMA

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Gliomas are the most frequently occurring primary malignancies in the central nervous system, and glioblastoma (GBM) is the most common and aggressive of these tumors. Protein kinase CK2 is a serine/threonine kinase composed of two catalytic subunits (alpha and/or alpha') and two beta regulatory subunits, which phosphorylates over 300 substrates. CK2 is a key suppressor of apoptosis, promotes angiogenesis, and enhances activation of the NF-kappaB, PI3K/AKT, Wnt and Notch signaling pathways. Elevated CK2 expression/activity has been reported in many tumors, including GBM, and it has been suggested that CK2 is essential for cancer cell survival. We have recently discovered that CK2 is a novel interaction partner of the Janus Kinases JAK1 and JAK2, and is required for activation of the JAK/STAT-3 pathway. Aberrant activation of signaling pathways including NF-kappaB, PI3K/AKT and JAK/STAT-3, has been implicated in tumor progression in GBM, and since CK2 is involved in their activation, we evaluated the expression and function of CK2 in the context of this cancer. Analysis of 537 GBMs from The Cancer Genome Atlas Project indicated the CSNK2A1 gene, encoding for CK2alpha, is frequently amplified in GBM (33.7%), which is significantly associated with the classical subtype of GBM. Inhibition of CK2 activity by pharmacological inhibitors (CX-4945) or knockdown of CK2 expression suppresses activation of the JAK/STAT, NF-kappaB and AKT pathways in primary human GBM xenograft cells. CK2 inhibitors decrease the adhesion and migration of GBM cells, in part through inhibition of integrin betal and integrin alpha4 expression. CK2 inhibitors also suppress growth, colony formation and cell cycle progression of GBM cells, and induce apoptosis of these cells. In vivo, CX-4945 significantly inhibits tumor growth and promotes survival in mice with intracranial human GBM xenografts by suppressing numerous signaling pathways. Therefore, CK2 inhibitors may be considered for treatment of patients with GBM.

CB-007. FGFR4 INHIBITION IMPACTS ON GLIOBLASTOMA AGGRESSIVENESS IN VITRO AND IN VIVO

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Fibroblast growth factors (FGF) and their high-affinity transmembrane receptors (FGFR1-FGFR4) represent a complex signal network regulating embryonic development and tissue homeostasis. In several cancer types, FGF/FGFR signal loops are deregulated by diverse genomic and epigenetic mechanisms consequently supporting cancer cell proliferation and survival. In case of human glioblastoma cell lines (N = 8) and primary cell cultures from clinical samples (N = 26) we found a widespread expression of several FGFs (like FGF1, FGF2, and FGF5) but also a significant overexpression of FGFR1 and FGFR4. Regarding FGFR1, all glioma cell models investigated additionally expressed the mesenchymal and more oncogenic splice variant FGFR1-IIIc. Consequently, we focused in this study on the consequence of FGFR4 as compared to FGFR1 inhibition on human glioblastoma models in vitro and in vivo. Application of the FGFR inhibitors (BIBF1120, ponatinib) as well as expression of dominant-negative versions of FGFR1 and FGFR4 significantly reduced in vitro cell growth and clonogenicity in the tested glioma cell models whereby dnFGFR1 tended to be more efficient as dnFGFR4. Accordingly, both dominant-negative FGFRs induced significant apoptosis whereby the effects of dnFGFR1 were again significantly stronger. Additionally, neurosphere formation, indicative for the presence of glioma stem cells, was profoundly reduced by both dnFGFRs. Interestingly, FGFR4 belonged to those genes significantly overexpressed in the cancer stem cell compartment (N = 16; mRNA expression arrays of neurosphere versus adherent cell culture). Surprisingly, the inhibitory effects on anchorage-independent growth in soft agar were opposite with significant mitigation by dnFGFR1 but almost complete blockade by dnFGFR4 in all glioblastoma models analysed. Accordingly, growth of human glioblastoma xenografts (N = 2) in SCID mice was completely inhibited by dnFGFR4 while only retarded by dnFGFR1. Summarizing our data substantiates a significant contribution of FGF/FGFR-mediated signals to different aspects of glioblastoma aggressiveness and suggests especially FGFR4 as potential target for therapeutic interventions.

CB-008. MicroRNA-1, DOWN-REGULATED IN GLIOMA, MODULATES THE CONTENT OF EXOSOMES RELEASED BY GLIOMA CELLS, MITIGATING THEIR PRO-ONCOGENIC POTENTIAL

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Glioblastoma multiforme (GBM) is the most common and aggressive intrinsic primary brain tumor in adults. GBM presents unique challenges to therapy due to its location, aggressive biological behavior and diffuse infiltrative growth. There is a recognized need for new approaches based on increased understanding of the biological nature of these tumors. Recent important developments include the discovery of microRNAs (miRs) and exosomes and their link with tumor microenvironment. MicroRNAs (miRs) are small, single stranded, non-coding RNAs that act as key regulators of gene expression, typically by reducing translation of target mRNAs with partial complementarity in their 3′-untranslated regions (UTRs). MiRs deregulation is a general feature of cancer, including GBM, and has been associated with tumor suppressor and oncogenic activities. Exosomes are cell-derived vesicles present in biological fluids and cultured medium of cell cultures. Exosomes are taken up by recipient cells in cell culture and in tumor microenvironment and they affect the phenotype of recipient cells by delivery of mRNAs, proteins, microRNAs, noncoding RNAs and DNA. We report that exosomes shed by donor glioma cells cultured as neurospheres alter the oncogenic features of recipient glioma cells by increasing their neurosphere formation potential. Exosomes also intensify tube formation capability of brain micro vesicular endothelial cells. We show that exosomes produced by donor cells stably or transiently overexpressing microRNA-1 (miR-1) commonly down-regulated in glioma, have significantly reduced tumorigenic ability. Mass-spec analysis showed that content of AnnexinA2 (ANXA2), normally abundant in exosomes, is significantly reduced in exosomes produced by miR-1 expressing cells and was verified as a direct target of miR-1. We conclude that replacement of miR-1 expression in glioma cells mitigates their exosome-mediated pro-oncogenic rearrangement of tumor microenvironment.

CB-009. SUBTYPE-SPECIFIC EXPRESSION OF THE TRUNCATED NEUROKININ-1 RECEPTOR IN GLIOBLASTOMA MULTIFORME

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Considerable evidence suggests that targeting the neurokinin-1 receptor (NK1R) is a promising approach to inhibit glioblastoma multiforme (GBM). Interestingly, recent data indicate that the truncated variant of NK1R, which ends at amino acid 311 (NK1RΔ311), is oncogenic in breast and colon cancers. However, the presence and expression pattern of NK1RΔ311 in GBMs have yet to be examined. Therefore, using a quantitative PCR

approach, the expression of NK1RΔ311 was examined in 20 GBM xenograft lines established at Duke's Preston Robert Tisch Brain Tumor Center. We found that NK1RΔ311 is variably expressed in these xenograft lines. Additionally, the xenografts were classified according to the four GBM subtypes defined by The Cancer Genome Atlas (TCGA) and found to have the following distribution: 8 classical, 8 mesenchymal, 4 proneural, and 0 neural. Further, the expression of NK1RΔ311 was found to vary significantly among subtypes ($p = 0.0402$), with mesenchymal and proneural tumors expressing higher levels than classical samples. Alternatively, expression of the full-length form of NK1R was not found to vary among subtypes ($p = 0.8561$). These findings pave the way for preclinical/clinical studies to test the efficacy of NK1R inhibition, either alone or in combination with other agents, in a subset of GBMs expressing elevated levels of NK1RΔ311.

CB-010. VARIABLE EXPRESSION OF THE TRUNCATED NEUROKININ-1 RECEPTOR IN GLIOBLASTOMA MULTIFORME

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While considerable evidence suggests that the neurokinin-1 receptor (NK1R) is a promising target for glioblastoma multiforme (GBM) inhibition, recent data has highlighted the role of a truncated splice variant of the receptor. This variant, which ends at amino acid 311 and is known as NK1RΔ311, has been implicated in both breast and colon cancers. However, the presence and expression pattern of NK1RΔ311 in GBMs have yet to be examined. Using a quantitative PCR approach, we examined the expression of NK1RΔ311 in 25 primary GBMs. We found that NK1RΔ311 is variably expressed in these primary tumors, with 11 samples (44%) exhibiting expression levels higher than a whole brain reference sample. Higher expression of NK1RΔ311 in a large percentage of GBMs, in conjunction with the data on breast and colon cancers, suggests a potentially oncogenic role for the protein in a subset of GBMs. In conclusion, the data presented here provides the first demonstration of the expression of NK1RΔ311 in GBM and underscores a potential role for this form of NK1R in GBM growth.

CB-011. GAMMA-GLUTAMYL TRANSFERASE 7 ACTS AS A TUMOR SUPPRESSOR IN GBM AND CORRELATES WITH GOOD PROGNOSIS

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Gamma-glutamyl transferase 7 (GGT7) is a part of a family of 13 enzymes that play a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and drug and xenobiotic detoxification. The GGT family has been shown to modulate crucial redox sensitive functions such as antioxidant/antitoxic defense and cellular proliferative/apoptotic balance making it an important player in proliferation and cell maintenance. Recent reports demonstrate that the GGT family has a role in tumorigenesis by regulating cell growth, invasion, and drug resistance. GGT7 is a novel GGT family member and it is hypothesized that it plays a role in the regulation of leukotriene synthesis, glutathione metabolism or gamma-glutamyl transfer. Recent reports have demonstrated that glioblastoma (GBM) patients have decreased expression of GGT7, suggesting that loss of GGT7 may enhance glial tumor growth. Analysis of the Rembrandt database demonstrated that GBM patients with lower expression of GGT7 had a worse prognosis ($P < 0.05$) than patients with higher expression. To determine if GGT7 is involved in GBM tumorigenesis we modulated GGT7 expression in GBM cell lines. We determined that under normal growth conditions exogenous expression of GGT7 resulted in a 2 -6 fold decrease in proliferation and 2- to 3-fold reduction in anchorage independent growth. Surprisingly, we observed a more pronounced reduction in GBM growth under starvation conditions, yielding a 10-fold decrease in proliferation and 3- to 4- fold in anchorage independent growth. Correspondingly, when endogenous GGT7 was reduced 5-fold using short interfering RNA (siRNAs) we found that cell growth was increased 3- to 10 fold and anchorage independent growth 2-3 fold. Together, our study suggests that GGT7 may act as a tumor suppressor in glial tumorigenesis and further analysis will be conducted to determine if GGT7 can be used a biomarker or therapeutic target for GBM.

CB-012. GENOME-WIDE shRNA SCREEN REVEALED SYNTHETIC LETHAL INTERACTION BETWEEN DOPAMINE RECEPTOR D2 (DRD2) AND EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) INHIBITION IN GLIOBLASTOMA

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INTRODUCTION: Targeted therapy in the treatment of glioblastoma has achieved little therapeutic gain, in large part due to the inherently redundant and dynamic molecular circuitry that drives glioblastoma proliferation. The available data suggest that simultaneous inactivation of critical nodes within this network will be required for meaningful clinical efficacy. METHODS: To identify such critical nodes, we conducted a genome-wide shRNA screen in search of gene silencings that exhibit synthetically lethal interactions with EGFR inhibition. RESULTS: The screen revealed that genes required for Dopamine Receptor D2 (DRD2) signalling serve essential functions in glioblastoma cells. Silencing of DRD2 by independent si- and sh-RNA compromised glioblastoma survival. This effect was rescued by expression of an RNAi resistant construct of DRD2 both in vitro and in vivo. DRD2 was overexpressed in human glioblastoma specimens relative to matched normal cortex. Importantly, clinical pathologic correlation revealed that high expression of DRD2 was associated with poor overall survival, supporting the thesis that DRD2 expression enhanced glioblastoma growth. This growth effect of DRD2 was mediated through the inhibitor G protein (GNAI2) to ERK1/2, where DRD2 and EGFR signalling converge. Synergy in tumoricidal activity was observed when combining haloperidol, a FDA approved drug that functions as an inhibitor of DRD2, and the EGFR inhibitor, AG1478, in both in vitro and in vivo glioblastoma models. CONCLUSION: DRD2 and EGFR signalling constitute two critical nodes in driving glioblastoma proliferation through ERK1/2. Simultaneous inhibition of these pathways through combination of the FDA approved anti-psychotic agent, haloperiodal, and EGFR inhibition constitute a sound strategy for glioblastoma therapy.

CB-013. CADHERIN-11 REGULATES MOTILITY IN NORMAL CORTICAL NEURAL PRECURSORS AND GLIOBLASTOMA

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Metastasizing tumor cells undergo a transformation that resembles a process in normal development when non-migratory epithelial cells modulate the expression of cytoskeletal and adhesion proteins to promote cell motility. Here we find a mesenchymal cadherin, Cadherin-11 (CDH11), is increased in cells exiting the ventricular zone (VZ) neuroepithelium during normal cerebral cortical development. When overexpressed in cortical progenitors in vivo, CDH11 causes premature exit from the neuroepithelium and increased cell migration. CDH11 expression is elevated in human brain tumors, correlating with higher tumor grade and decreased patient survival. In glioblastoma, CDH11-expressing tumor cells can be found localized near tumor vasculature. Endothelial cells stimulate TGFβ signaling and CDH11 expression in glioblastoma cells. TGFβ promotes glioblastoma cell motility, and knockdown of CDH11 expression in primary human glioblastoma cells inhibits TGFβ-stimulated migration. Together, these findings show that Cadherin-11 can promote cell migration in neural precursors and glioblastoma cells and suggest that endothelial cells increase tumor aggressiveness by co-opting mechanisms that regulate normal neural development.

CB-014. THE COUPLING AND FUNCTION OF ADHESION RECEPTOR GPR124 IN GLIOBLASTOMA MULTIFORME

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GPR124 is an orphan receptor that belongs to the Adhesion GPCR family. Members of this family are characterized by long N-terminal segments that contain many of the same functional domains found in cadherins, integrins, and tyrosine kinases, but are typically absent in GPCRs belonging to other families. While little is known about adhesion GPCRs, recent studies show

that GPR124 plays a role in endothelial cell migration and differentiation during angiogenesis. We found that GPR124 mRNA is highly expressed by various human Glioblastoma Multiforme (GBM) cell lines. Due to the high expression of GPR124 in GBM cells and its known role in cellular migration and differentiation, we hypothesize that these orphan receptors control the migration and proliferation of GBM cells through specific signal transduction pathways and thus represent promising new therapeutic targets to treat this cancer. To test our hypothesis, we measured cell migration using a modified Boyden chamber assay that was developed in our laboratory. We found that GPR124 over-expression increases the migration of U87MG cells stimulated by lysophosphatidic acid (LPA), and also increases the rate of proliferation of these cells. Conversely, stable knockdown of GPR124 expression by shRNA in T98G cells reduces their ability to migrate, and reduces their proliferation rate in vitro. To elucidate the signaling pathway involved in GPR124 biology in GBM, we used a proteomics approach to determine the effector proteins that couple to this protein. Myc-tagged GPR124 was heterologously expressed in U87MG and the proteins associated with these receptors were analyzed by Stable Isotope Labeling of Amino Acids in Cell Culture (SILAC) mass spectrometry. We found a concise list of effector proteins that are known to control second messenger signaling and cell migration. Together, our results suggest that GPR124 is expressed by GBM and tightly regulates how these cells proliferate, and migrate.

CB-015. CADHERIN-MEDIATED CHANGES IN RADIOSENSITIVITY AND EXPRESSION OF CD133 AND LEF-1 IN U373 GLIOBLASTOMA CELLS

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BACKGROUND: Mounting pathological data exist supporting the impact that cadherins may have in metastatic potential and prognosis of various cancers. With regard to gliomagenesis and progression, there is a paucity of mechanistic data regarding the role of members of the cadherin superfamily. Previously, we have demonstrated that a cadherin-selective adhesion substrate promotes the migratory capacity of high grade glioma cells in culture. Here, we examine the role cadherin-based adhesion and associated Wnt-signaling with respect to radiation (XRT) sensitivity. METHODS: Using the U373 (Upsalla) cells, we exposed cultures to a recombinant cadherin fusion protein, containing the full ectodomain of C-cadherin alone or with XRT. Proliferation and viability were assessed via the MTT assay, while changes in gene expression were analyzed by quantitative PCR. RESULTS: At 24 hours following radiation treatment, cultures treated with high-dose (1 μg/ml) cadherin ectodomain had increased survival compared to no treatment controls ($p = 0.05$), while at 72 hours, both high-dose and low-dose (100 ng/ml) groups had significant survival advantages over controls ($p = 0.02$, $p = 0.036$, respectively). Gene expression data show a two-fold decrease in CD133 expression in the cadherin-ectdomain group with a return to control baseline expression following 4 Gy. Similarly, E-cadherin gene expression was decreased two-fold in the ectodomain-only treated group, with return to baseline following XRT. Beta-catenin expression was constant, while LEF-1 expression was increased two-fold and three-fold in the cadherin alone and cadherin + XRT groups, respectively. CONCLUSIONS: Cadherin-mediated adhesion is capable of increasing the relative radioresistance of high grade glioma cells in culture and is associated with a significant decrease in CD133 expression in the absence of radiation. Increased LEF-1 expression following cadherin-ectodomain exposure alone or with radiation, implicates the Wnt signaling pathway. These data support the exploration of Wnt pathway and its components as therapeutic targets for radiosensitization in gliomas.

CB-016. ROLES OF NHE-1 ACTIVATION IN GLIOBLASTOMA CANCER CELL MIGRATION AND SURVIVAL IN COMBINATION WITH TEMOZOLOMIDE

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Sodium-hydrogen exchanger isoform 1 (NHE-1) is ubiquitously expressed and regulates intracellular pH (pH_i) and extracellular microdomain (pH_e) homeostasis. NHE-1 was shown to play an important role in promoting survival and migration/invasion of cancer cells. However, whether NHE-1 activity affects glioma cell migration and survival, and the effects of temozolomide (TMZ) chemotherapy remain unknown. In this study, we detected increased NHE-1 protein expression in primary glioma cell lines (GC#22 and GC#99) compared to control human neural stem cells and astrocytes. NHE-1 protein was localized at the lamellipodia of GC#22 via immunofluorescence staining. NHE-1 activity is involved in regulating GC resting pH_i. An alkaline pH_i (7.39 ± 0.04) was detected in GC cells. Pharmacological inhibition of NHE-1 with HOE 642 acidified cells (7.21 ± 0.06, $p < 0.05$). GC pH_i was also decreased (7.15 ± 0.07, 7.26 ± 0.03, respectively) after 2 or 4 h TMZ (100 μM) treatment. But, NHE-1 blockade with HOE 642 further acidified GC (6.78 ± 0.11, and 6.88 ± 0.02, respectively), suggesting intact NHE-1 function after TMZ treatment. Moreover, GC mobility and migration were increased in the presence of TMZ. HOE 642 significantly decreased the TMZ-associated glioma cell mobility and migration. Most importantly, we found that a combined treatment with TMZ and HOE 642 enhanced glioma cell apoptosis (detected by cleaved caspase-3 expression) compared to TMZ treatment alone. Therefore, our study shows that 1) NHE-1 protein is important in maintaining an alkaline resting pHi in glioma cells; and 2) inhibition of NHE-1 activity suppresses glioma cell migration and augments TMZ-induced apoptosis. These findings suggest that the novel strategy of adding NHE-1 blockade may increase the efficacy of TMZ chemotherapy and improve clinical outcomes.

CB-017. pDING ENHANCES TEMOZOLOMIDE-INDUCED GLIOBLASTOMA CELL GROWTH INHIBITION VIA miR-21 DOWN REGULATION

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INTRODUCTION: Introduction: pDING is a 38 kDa protein (p38SJ) derived from the Hypericum perforatum plant and possesses phosphatase activity known to inhibit cell cycle advancement at G1 via a variety of phosphorylated proteins, including ERK1/2 and AKT. We examined the effects of pDING exogenous treatment on Gli1 primary glioblastoma cells, a radioresistent cell line, in culture to evaluate its efficacy as an adjunct to conventional therapy in resistant glioblastoma tumors; we also examined alterations in the miRNA levels of this cell line in order to further elucidate the mode of action for pDING's antitumor growth activity. METHODS: Gli1 primary glioma cell lines were treated with 20 μM of temozolomide, 0.6 μg/ml of pDING, 25 μJ of UV radiation, and dual and triple combinations of these therapies. Cells counts were tracked daily to evaluate growth. miRNA fold regulation was calculated using quantitative RT-PCR using miScript miRNA PCR arrays and total RNA isolates from each sample and from YFP and YFP-p38SJ transfected Gli1 cells. RESULTS: pDING treatment created 40-50% reductions in Gli1 cell growth in all treatment arms, in agreement with previous reports. pDING treatment increased temozolomide-mediated cell growth inhibition by 6% when applied in combination to Gli1 cells. No additive effect was noted with UV radiation, and no sustained growth inhibition was noted with UV radiation alone, indicating that pDING provides some concerted antitumor abilities with alkylating chemotherapy but is insufficient to sensitize radio-resistant glioblastoma cell lines. RT-PCR demonstrated a 2.23 fold down regulation of miR-21 and a 2.21 fold down regulation of miR-125. These miRNA have been associated with AKT up regulation and mitochondria mediated apoptotic pathways, respectively. These results suggest that pDING-mediated cell cycle inhibition and pro-apoptotic properties may be in part affected through miRNAs down regulation in addition to the direct phosphorylation of cell cycle-related proteins.

CB-018. TRANSCRIPTIONAL REGULATION AT IGF2 PROMOTERS AND MECHANISTIC INSIGHTS ON INDUCTION OF IGF2 DOWNSTREAM OF YAP IN Shh MEDULLOBLASTOMAS

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In mouse models for Shh-medulloblastomas, IGF2 is required for tumor formation, growth, and metastases. We showed that YAP over-expression induces IGF2 expression as a part of YAP's radiation-resistance program in mouse Shh-medulloblastomas and in cerebellar granule neuron precursors (CGNPs), proposed cells-of-origin for the Shh subclass of medulloblastoma. IGF2 and its regulatory program may represent a therapeutic target in

medulloblastoma, but the mechanism of IGF2 induction downstream of YAP is not well understood. The anomalous loss of IGF2 imprinting in the human fetal brain is intriguing and exemplifies the complexity of the IGF2 gene's regulation. Although CTCF mediates allele-specific expression at the IGF2/H19-imprinted locus in both mice and humans, subsequent evidence suggests that CTCF binding at the IGF2/H19 imprinting control region is insufficient to regulate IGF2/H19 expression in human tissues. This makes a compelling case in favor of studying the transcriptional regulation at the different IGF2 promoters in order to delineate the mechanism of IGF2 induction downstream of YAP. We have employed biotinylated-DNA 'fishing' combined with proteomics to delineate the transcriptosomes at the IGF2 promoters in medulloblastoma cells, and directly validated them using medulloblastoma cell-derived material. The results of transcriptosome analyses revealed factors, Yb1 and Myef2, associated with IGF2 promoter Pr3 in PZP53 cells (derived from a Ptc + /− /p53 − /− mouse medulloblastoma) and SmoA1 tumor tissue. Myef2 was consistent in its association with IGF2 promoter Pr3 in mouse P5 cerebella, as in case of PZP53 cell line and SmoA1 tumor tissue. Of note, we observed increased levels of Myef2 and Yb1 in Shh-treated CGNPs. Our results indicate that association of Yb1 at IGF2 promoter Pr3 could be mediating the induction of IGF2 downstream of YAP and the radiation-induced expression of IGF2 could be linked to DNA repair mechanisms of Yb1.

CB-019. INSIGHTS INTO THE MOLECULAR MECHANISM OF PID1 GROWTH-INHIBITORY EFFECTS IN GLIOMAS

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Phosphotyrosine interaction domain containing 1 (PID1) is a recently identified gene which inhibits insulin-mediated signaling in adipocytes and muscle cells. As we reported at prior meetings, using six independent datasets we find that less favorable medulloblastomas (Group 3, Group 4, Anaplastic) and gliomas (glioblastoma multiforme, GBM) had lower PID1 mRNA compared to more favorable medulloblastomas and gliomas. Moreover, higher PID1 mRNA correlates with better patient outcome both in medulloblastoma and glioma patients. Moreover, PID1 overexpression in brain tumor cell lines exerted growth-inhibitory effect in glioblastoma and embryonal brain tumor cell lines (medulloblastomas, ATRT), suggesting a possible causal role for it in modulating tumor aggressiveness. PID1 harbors a phosphotyrosine binding (PTB) domain, hypothesized to interact with proteins harboring the consensus PTB binding sequence NPXY. A potential binding partner is LRP1 (low density lipoprotein receptor-like protein 1), a plasma membrane receptor involved in signaling, lipid homeostasis, migration and survival, and clearance of apoptotic cells. LRP1 harbors an NPXY motif in its cytoplasmic tail. We find that immunoprecipitation of native LRP1 in three GBM cell lines using two different antibodies to LRP1 was able to coimmunoprecipitate native PID1. To determine if the PID1 growth-inhibitory effect was indeed, mediated via its PTB domain, we generated deletion mutants of PID1 that either lacked the PTB domain or expressed only it. Using colony formation assays we find that while the PID1 mutant harboring the PTB domain indeed mediated inhibition in absence of other PID1 domains, the other domains of PID1 caused similar inhibition. Thus, we concluded that PID1 inhibition of glioma and medulloblastoma cells was mediated via several domains in PID1.

CB-020. CDK2-MEDIATED OLIG2 PHOSPHORYLATION REPRESSES p27 EXPRESSION AND PROMOTES BRAIN TUMOR DEVELOPMENT

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Oligodendrocyte transcription factor 2 (OLIG2) is an important proneural factor regulating early neural development and promotes proliferation of neural progenitors and glioma. However, how OLIG2 is regulated, thereby executing its cellular functions is largely unknown. Here we identified OLIG2 as a critical phosphorylation target for cyclin-dependent kinase 2 (CDK2). CDK2 directly interacts with and phosphorylates OLIG2 at Ser14. The OLIG2 phosphorylation by CDK2 is accompanied with cell cycle progression from late G1 through S phase and is correlated with proliferative marker Ki-67. Phosphorylated Olig2 exerts pro-proliferative effects that are reflected in glioma cells and in murine xenograft models of glioma. We show that bFGF stimulation regulates OLIG2 phosphorylation and a decline of OLIG2 phosphorylation was observed in differentiating neural progenitors. CDK2-mediated OLIG2 phosphorylation stabilizes OLIG2 protein from proteasomal degradation. We also propose p27/KIP1 as a potential gene repressed by OLIG2. OLIG2 phosphorylation is required for OLIG2 to bind to the p27 promoter regions to repress p27 transcription thereby promoting cell cycle progression and cell proliferation. Phosphorylated OLIG2 binds to the E-Box regions of p27 promoter and represses p27 gene transcription, which in turn activates CDK2 in positive feedback manner. p27 antagonizes Olig2 phosphorylation and inhibits tumor cell proliferation and tumor development. Further, The Cancer Genome Atlas (TCGA) data analysis showed that OLIG2 co-expresses with CDK2 in GBM subclasses. Importantly, OLIG2-high glioma initiating cells are highly sensitive to CDK2 inhibitor treatment, indicating that OLIG2 plays an instrumental role in mediating CDK2-regulated glioma initiating cell growth; OLIG2 expression levels can be a promising biomarker for selection of GBM patients with high OLIG2 expression for CDK2 inhibitor treatment. In conclusion, our findings provides a molecular mechanism underlying the regulation of OLIG2 and OLIG2-regulated brain tumor development, which may improve the efficacy of treatment of GBM patient with personalized therapy by CDK inhibitors.

CB-021. MUTANT EGFR SUPPRESSION OF microRNA-9 INDUCES FOXP1 TO ENHANCE GLIOBLASTOMA TUMORIGENICITY

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Glioblastoma (GBM), the most common primary malignant brain tumor, frequently displays amplification and/or mutation of the epidermal growth factor receptor (EGFR) gene. Highlighting the importance of EGFR in the pathogenesis of GBM, aberrant EGFR signaling is required for GBM maintenance, growth, invasion and resistance to therapeutic agents. We hypothesized that the oncogenic pathophysiology exerted by EGFR requires the modulation of microRNA (miR) activity. To test this hypothesis, we compared the miR profiles of GBM cells with activated wild type EGFR (wtEGFR) and mutant EGFR (ΔEGFR) to cells with non-activated wtEGFR or kinase dead ΔEGFR. We observed and validated that ΔEGFR suppresses miR-9. The repression of miR-9 is due to the negative regulation of the primary miR-9 encoding transcript, pri-miR-9-2, by ΔEGFR. Downstream of ΔEGFR, the Ras/PI3K/AKT axis was required to suppress miR-9. We identified the transcription factor, FOXP1, to be a bona-fide miR-9 target. Upregulation of miR-9 decreased expression of FOXP1 in ΔEGFR cells, suggesting that miR-9 and FOXP1 may regulate, in part, ΔEGFR-dependent GBM growth. Consistent with this hypothesis, miR-9 antagonized the tumor growth advantage conferred by ΔEGFR while FOXP1 knock-down inhibited the growth of ΔEGFR-driven tumors. Upregulation of FOXP1, as a consequence of inhibiting miR-9 activity, increased the tumorigenicity of GBM cells, suggesting that miR-9 is a tumor suppressor while FOXP1 likely functions as an oncogenic factor in GBM. Finally, high FOXP1 expression was significantly associated with poor survival in GBM patients, further supporting the notion that FOXP1 is an oncogenic factor. Collectively, these data reveal a novel regulatory mechanism by which ΔEGFR suppression of miR-9 upregulates FOXP1 to increase tumorigenicity.

CB-022. EFFECTS OF EGFR SIGNALING ON THE MODE OF DIVISION OF NEURAL PROGENITORS AND GLIOMA PRECURSORS

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Dysregulation of growth factor receptor tyrosine kinase (RTK) signaling pathways is very common in malignant glioma including glioblastoma multiforme (GBM). The intra-tumoral heterogeneity of GBM extends to RTK alterations (Snuderl et al, Cancer Cell; 2011; 20; 810-817; Szerlip et al, PNAS; 2012; 109; 3041-3046) and provides a rationale for multimodal therapies (Dunn et al, Genes Dev; 2012; 26; 756-784). Recent evidence suggests that oligodendrocyte precursor cells (OPCs) may act as cellular origin of astrocytomas (Chen et al, Cell; 2012; 149; 36-47). How RTK dysregulation and anti-RTK therapies affect non-neoplastic OPC as well as astrocytoma precursor is not fully understood. OPCs self-renew and generate differentiating oligodendrocytes by undergoing asymmetric divisions. Only recently, we learned that the epidermal growth factor receptor (EGFR) participates in asymmetric OPC division (Sugiarto et al, Cancer Cell; 2011; 20; 328-340). Here, we investigated in further detail the molecular and biological changes that occur as non-neoplastic OPCs turn into astrocytoma precursors due to dysregulated RTK

signaling. Amongst other approaches, we forced expression of either EGFRvIII, a frequent and constitutively activating mutation of EGFR, or wild-type EGFR, in Ink4a/Arf deleted OPCs isolated from young adult mice. We will discuss defects associated with these alterations in particular with regards to changes in progenitor properties such as asymmetric division. We will further show analyses of the molecular and cellular changes on OPCs and astrocytoma precursors caused by RTK pharmacological inhibition. Our studies are expected to bring further insights into role of RTK signaling in neural progenitors and astrocytoma precursors. We expect to bridge the gap in our understanding of the effects of RTK mono- and combination therapy on neural progenitors. We may find novel defects downstream of RTK dysregulation, which when targeted pharmacologically, may effectively eliminate malignant cells.

CB-023. ALTERNATIVE SPLICING IN GLIOBLASTOMA: A BIG NEW WORLD AHEAD

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Alternative splicing plays a key role in determining tissue-specific differentiation patterns. The emerging evidence places alternative splicing in a central position between transcription and translation, in that it can respond not only to various signalling pathways that target the splicing machinery but also to transcription factors and chromatin structure. Changes in splicing have been implicated in numerous pathologies including cancer or neurodegenerative diseases. The objective of our study was to determine whether aberrant alternative splicing could play a role in the malignant phenotype of GBM. Patients with GBM were operated with 5-aminolevulinic fluorescence guided surgery. The fluorescent was used to take biopsies from the tumor center, and from adjacent normal-looking tissue. Three paired normal/GBM samples were analyzed in a HJAY J array. We validated our results with conventional PCR and qRT-PCR in those tumor samples and in ten additional GBMs. We performed functional studies using MTT assays (proliferation) and IF qRT-PCR (differentiation). We generated a list of seven genes with differential alternative splicing between central tumor samples and the adjacent normal-looking tissue. DPF-2(BAF45d) was one of our top candidates. Interestingly, DPF-2 is known as a tumor suppressor gene in the context of the SWI/SNF complex and plays a key role in the development of the central nervous system. The inhibition of our DPF-2 isoform resulted in a significative reduction in proliferation and in a morphology changes towards a more differentiated phenotype in GBM cell lines. Interestingly, our DPF2 tumoral isoform was the predominant transcript in early postnatal murine neural precursors and its expression disappeared as these cells were instructed towards neurons. Our results suggest that the alternative splicing of DPF-2 in GBM could participate in the maintenance of an undifferentiated cellular state. In addition, our data suggest that alternative splicing is a mechanism that could be central to GBM development.

CB-024. AN Ehsp90-Lrp1 AXIS REGULATES Epha2 SIGNALING AND CELL MORPHOLOGY

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Our goal is to understand factors contributing to the deadly invasive and infiltrative spread of glioma. The EphA2 receptor is overexpressed in gliomas and many other solid tumors, and is required for glioma cell motility and invasion. Extracellular Hsp90 (eHsp90) is also known to promote cell motility and invasion in tumor models. We recently demonstrated that eHsp90 plays an essential role in glioma tumor cell invasion via signaling with its receptor LRP1. We reported that eHsp90 facilitates interaction of EphA2 with LRP1, an event required for glioma cell motility and invasion. We herein investigate the mechanisms by which eHsp90 and LRP1 may regulate EphA2 signaling function. The EphA2 ligand ephrinA1 is suppressed in cancers and blocks EphA2 driven cell motility. Interestingly, ephrinA1 disrupted EphA2 interaction with LRP1 and facilitated its interaction with c-cbl concomitant with receptor internalization and subsequent Rho-mediated cell rounding, hallmarks of ephrinA1-mediated signaling events. Thus, the ability of ephrinA1 to facilitate EphA2 interaction with an LRP1-deficient protein complex coincides with its suppression of cell motility. Surprisingly, we report that eHsp90-LRP1 signaling is also required for ephrinA1-dependent signaling and internalization, indicating that eHsp90-LRP1 plays a critical role in coordinating both surface and internalized receptor populations. This is the first report to demonstrate a role for eHsp90-LRP1 as regulators of receptor internalization, and may point to a broader role for these proteins in regulating glioma receptor signaling, invasion, and dissemination.

CB-025. COORDINATE ACTIVATION OF Shh AND PI3 KINASE SIGNALING PATHWAYS IN PTEN-DEFICIENT GLIOBLASTOMA PRESENTS NEW OPPORTUNITIES FOR TARGETED THERAPY

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In glioblastoma, PI3 kinase signaling is frequently activated by loss of the tumor suppressor PTEN. However, it is not known whether inhibiting PI3 kinase represents a selective and effective approach for treatment. Here we interrogate large tumor databases and find that Shh signaling is activated in PTEN-deficient glioblastoma. We demonstrate that Shh and PI3K pathways synergize to promote tumor growth and viability in human PTEN-deficient glioblastomas. A combination of PI3K and Shh signaling inhibitors not only suppresses activation of both pathways, but also abrogates S6 kinase signaling. Accordingly, simultaneously targeting both pathways results in mitotic catastrophe and tumor apoptosis, and dramatically reduces growth of PTEN-deficient glioblastomas in vitro and in vivo. The drugs tested here appear safe in humans; therefore this combination may provide new targeted treatment for glioblastoma.

CB-026. NEW ALKYLINDOLES SELECTIVELY PROMOTE APOPTOSIS IN GBM CELLS INDEPENDENT OF CANNABINOID CB_1 AND CB_2 RECEPTORS

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Grade IV astrocytomas, or glioblastoma multiform (GBM), are the most common, malignant, and deadly primary brain tumors. Recent evidence points towards cannabinoids as potent anti-tumorigenic agents. Cannabinoid agonists stimulate cannabinoid CB_1 and CB_2 receptors and regulate key processes of GBM tumorigenesis, including cell differentiation, viability, and migration. We developed a novel alkylindole compound, JWH-451, using the potent cannabinoid agonist WIN55,212-2 as a scaffold and found that it promotes apoptosis in several GBM cell lines independent of CB_1 and CB_2 receptors, suggesting a novel mechanism of action for these compounds. Here we tested the therapeutic value of two second generation compounds, ST-25 and ST-34. Both compounds dose-dependently killed a panel of 8 human astrocytoma cell lines, including GBM8. We then tested the *in vitro* therapeutic index of these compounds on T98g human astrocytomas and HepG2 cells, a human liver cell line commonly used to assess compound toxicity. While Temozolomide and Carmustine killed T98G and HepG2 cells with similar potencies, JWH-451, ST-25, and ST-34 demonstrated an enhanced potency on T98G cells compared with HepG2 cells, providing *in vitro* therapeutic indices of 4, 53, and 11, respectively. We found that these compounds decreased GBM cell proliferation and viability. Remarkably high concentrations of these compounds did not kill mouse neurons in primary culture and CD44+ astrocytes derived from human stem cells. With regard to the mechanism of action of JWH-451, ST-25, and ST-34, we found that these compounds increased phosphorylated polo-like kinase 1 (PLK-1), promote PARP cleavage, activate caspases, and thus promote apoptosis. Remarkably, the *in vivo* safety profile of JWH-451 was established at a maximal administrable dose, which reinforces a promising therapeutic index. Our *in vitro* and *in vivo* results support the promise of further testing of novel alkylindole compounds as GBM therapeutics and the further study of their mechanism of action.

CB-027. REIC/Dkk-3, ONE OF DICKKOPF (Dkk) FAMILY MEMBERS, CONTRIBUTES TO THE ANTI-TUMOR EFFECTS IN GLIOBLASTOMA THROUGH REGULATION OF BOTH Wnt SIGNAL PATHWAYS

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BACKGROUND AND PURPOSES: Glioblastoma multiforme (GBM) is a lethal primary brain tumor. Whether adenovirus-mediated REIC/Dkk-3 (Ad-REIC) inhibits the proliferation of these cells and how REIC/Dkk-3 protein regulates the Wnt signaling pathway remain to be elucidated. To verify the mechanisms underlying these effects we focused on the regulation by REIC/Dkk-3 protein on the interaction between the Wnt proteins Wnt3a and Wnt5a and their co-receptors LRP6 and ROR2 and examined the Wnt signal downstream cascade. METHODS: We used U87MG, U251MG, and TGB-111 cells and a murine U87MG cell xenograft model to compare the anti-tumor effects of Ad-REIC and Ad-LacZ. We analyzed the expression of Wnt proteins and its co-receptors by western blot and quantitative RT-PCR. Their interaction was examined by the immunoprecipitate and the activities of downstream cascade were examined by pull-down assay. RESULTS: Ad-REIC exerted anti-tumor effects in GBM cells and mice xenograft models. Notably, Wnt3a and Wnt5a interacted with both the co-receptors LRP6 and ROR2 in Ad-LacZ treated cells. Interestingly, the binding of Wnt3a to LRP6 and of Wnt5a to ROR2 was preferentially inhibited by REIC/Dkk3 in Ad-REIC treated cells. In the Wnt signal downstream, b-catenin but not Rac1 was reduced and RhoA activation was inhibited, while JNK and c-Jun were activated. This suggests that the regulation by REIC/Dkk-3 of both canonical and non-canonical Wnt signaling pathways upstream affects functional molecules downstream, thereby exerting anti-tumor effects in GBM. CONCLUSIONS: Ad-REIC gene therapy may have promise for treating GBM.

CB-028. THE IMMUNOMODULATORY EFFECTS OF DECORIN - A NOVEL AGENT FOR THE TREATMENT OF GLIOBLASTOMA MULTIFORME

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BACKGROUND: Current treatments for Glioblastoma (GBM) are inadequate and immunotherapeutics may provide better outcomes for patients. Human recombinant Decorin is a glycoprotein that modulates inflammation & GBM growth by inhibiting Transforming Growth Factor, Vascular Endothelial Growth Factor and Epidermal Growth Factor Receptor. As Decorin is found in human tissue rich in fibrillar collagen (e.g. skin, heart, bone lung & liver) it is a potentially safe drug to be trialled in humans. METHODS: The local immune and microglial response to intracerebral injections of Decorin was investigated. Rats (n = 6 for each group) received either an injection of PBS (control) or 5 μl 5mg/ml Decorin and were sacrificed at 30min, 3 & 24 hrs or after continuous infusion of either PBS (control) or Decorin for 7 days. Macroscopic evaluation of brain, lung, heart, liver, spleen, intestine and kidney was performed for signs of abnormalities. Blood and CSF samples were taken and analysed by ELISA or Luminex for presence of Decorin and change in inflammatory markers. Immunohistochemistry was also performed to investigate the effects of Decorin on microglia using OX42 and ED1 antibodies. RESULTS: There was no inflammatory reaction or cavity formation in brain. No end-organ damage was visible in brain, heart, lungs, kidney and intestines. Compared to PBS, Decorin supressed the microglial response after direct injections into the brain. At 30 mins, low levels of decorin were detected (with ELISA) in CSF and serum but none was detected at 3hrs and 24hrs after injection. CONCLUSIONS: Decorin showed no dose limiting toxicity, whilst maintaining its immunomodulatory effects in brain. These findings show a safe pharmacokinetic and toxicological profile of Decorin.

CB-029. MUTANT IDH1 SUPPRESSES PROSTATE APOPTOSIS RESPONSE-4 IN GLIOMAS

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Cancer-associated mutations in isocitrate dehydrogenase 1 (IDH1) alter its catalytic properties, leading to the production and accumulation of D-2-hydroxyglutarate (D-2-HG). Recent work has shown that this oncometabolite promotes hypermethylation and inhibits differentiation, but its full range of effects on gliomas and gliomagenesis remains unclear. We found that in vitro treatment with exogenous unmodified D-2-HG or stable expression of R132H IDH1 downregulates prostate apoptosis response-4 (Par-4), a protein known to induce cancer-selective apoptosis in a variety of in vitro, xenograft, and transgenic models. This downregulation occurs via Par-4 mRNA degradation, not promoter methylation, in U87MG glioma cells. IDH1-mutant glioblastomas (GBMs) from the Cancer Genome Atlas have lower Par-4 mRNA relative to wild-type tumors ($P < 0.01$), and Par-4 expression is markedly lower in IDH1-mutant high grade gliomas via immunohistochemistry ($P = 0.003$). Although IDH1 mutations are a well-known favorable prognostic marker in gliomas, among high-grade gliomas that are IDH1 wild-type, those that express Par-4 on initial resection correlate with significantly longer survival (median survival 18.4 versus 8.0 months, $P = 0.002$). These data suggest that a.) the effects of mutant IDH1 and D-2-HG on gliomas extend beyond direct gene hypermethylation, b.) suppression of Par-4 might contribute to IDH1-mutant tumorigenesis; c.) regardless of IDH1 status, Par-4 may be an effective sensitizer of gliomas to apoptosis.

CB-030. EFEMP1 ATTENUATES EGFR SIGNALING ACTIVITIES AND THE PROGNOSTIC EFFECT OF EFEMP1DEPENDS ON THE LEVEL OF EGFR EXPRESSION IN GLIOMAS

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In most clinical cancers, EFEMP1 is tumor suppressive, with widely occurring DNA hypermethylation, and a favorable prognostic effect of this gene's expression level on patient survival, including patients with glioblastoma multiformes (GBMs). However, an unfavorable prognostic effect of EFEMP1 was shown in cervical cancer and a tumor promoting effect was shown in pancreatic cell lines and glioma stem-like cell cultures. Our prior studies showed that EFEMP1 suppressed glioma cell expression of VEGFA and that restoring VEGFA rescued tumor onset but not tumor growth speed. Here we further explored EFEMP1's role in targeting EGFR-mediated activation of oncogenesic activates. We analyzed real-time qRT-PCR-derived expression values of EFEMP1 and EGFR (normalized to ACTB) for their association in overall survival (OS) of patients with gliomas (n = 166). Ignoring other factors (histology, age, and recurrent status), the effect of EFEMP1 on OS is significantly different between patients with low vs. high EGFR expression. In low EGFR gliomas, EFEMP1 is deleterious to OS, while in high EGFR gliomas, EFEMP1 is protective with regard to OS. This finding is consisitent in accordance with our experimental data demonstrating that EFEMP1 suppresses in vitro growth and orthotopic tumor formation by a glioma cell subpopulation expressing high level of EGFR, while lacking a tumor suppression effect on the syngeneic glioma cell subpopulation expressing a low level of EGFR. Using induced lentiviral vector to transiently express EFEMP1, we further showed EFEMP1's effect in blocking EGF-inducted EGFR signaling activities in three glioma cell lines (U251, LN229, and U87). Consistent with repeated EGF-like modules within the EFEMP1 molecule co-immunoprecipitation showed there is a direct interaction between EFEMP1 and EGFR in glioma cells. Overall, the data demonstrate an EGFR-related, cell-context dependent, function of EFEMP1 in malignant gliomas tumors.

CB-031. MICROGLIAL/BRAIN MACROPHAGE TOLL-LIKE-RECEPTOR 2 SIGNALING PROMOTES GLIOMA EXPANSION

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Malignant gliomas are the most frequent primary tumors of the brain with poor clinical prognosis. Infiltrating peripheral macrophages and resident microglia as the intrinsic immune competent brain cell contribute significantly to the tumor mass. We have previously shown that microglia/brain macrophages promote glioma expansion by up-regulating metalloprotease MT1-MMP through Toll-like receptor (TLR) and its adaptor protein MyD88. In this study we identified TLR2, as the main TLR controlling MT1-MMP expression and pro-tumorigenic signaling in microglia. Glioma-derived soluble factors and synthetic TLR specific ligands induced MT1-MMP expression in microglia from wild-type (WT) mice but not TLR2−/− mice. By using the organotypic brain slice model, we found that tumor expansion depended on both parenchymal TLR2 expression and the

presence of microglia. The implantation of mouse GL261 glioma cells into TLR2−/− mice resulted in significantly smaller tumors, reduced MT1-MMP expression, and enhanced survival rates as compared to WT control mice. TLR2 is also highly expressed in tissue from human gliomas (which contains microglia/brain macrophages) and inversely correlates with patient survival. In search for an endogenous TLR2 ligand released from glioma cells, we screened glioma conditioned medium by mass spectrometry and found versican, an extracellular matrix proteoglycan and a reported ligand of TLR2. Versican is highly up-regulated in gliomas but not in microglial cells. Versican silenced gliomas induced less MT1-MMP expression on microglia both in vitro and in vivo. Implanting versican silenced GL261 cells into mouse brain resulted in smaller tumors compare to the controls. Our results show that glioma released factors convert microglia/brain macrophages into a pro-tumorigenic phenotype through TLR2 signaling and thus TLR2 might be a novel target for glioma therapies.

CB-032. THE ROLE OF ARHGAP36 AS A NOVEL DRIVER IN HIGH-RISK HUMAN MEDULLOBLASTOMA

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INTRODUCTION: Medulloblastoma (MB) is the most frequent childhood malignancy of the CNS. Current trials aim to identify effective treatments with reduced side effects, but lack of knowledge regarding drivers prevents development of more precise therapies. MB is divided into four molecular subgroups based on gene expression, histology and developmental origin: Shh, Wnt, 3 and 4. Our lab identified a novel, non-Shh, non-Wnt driver of MB using Sleeping Beauty insertional mutagenesis. Rho GTPase Activating Protein 36 (Arhgap36) was the top candidate to emerge from this screen, showing insertion and overexpression in 14/22 MBs harvested. METHODS: Preliminary studies with qRT-PCR, tissue microarray and immunohistochemistry showed Arhgap36 overexpression in Group 3 and 4 human MB. We are employing multiple genetic techniques to elucidate the role of Arhgap36 in medulloblastomagenesis. We will overexpress Arhgap36 in NIH 3T3 cells and monitor the effects on cell signaling and Rho GTPase activation. We will also use TALENs to knockout Arhgap36 in human MB cell lines and assess the effects of Arhgap36 on cellular signaling through mass spectrometry and microWestern analysis. RESULTS: Constructs for Arhgap36 overexpression have been synthesized and transfected into NIH 3T3 cells for analysis. One human Group 3 MB line has shown an "oncogene-addiction" phenotype, as it was resistant to TALEN knockout of ARHGAP36. Knockout experiments have been repeated in the presence of a rescue, Tet-regulated cDNA to conditionally complement ARHGAP36 expression. CONCLUSIONS: Arhgap36 overexpression is strongly associated with MB, and our findings indicate that it may play a driving role in Group 3 and Group 4 medulloblastomagenesis. Furthermore, Arhgap36 may represent a novel target for therapeutic efforts aimed at treating patients with MB.

CB-033. A ROLE FOR MATRIX METALLOPROTEINASES IN INVASIVE AND MALIGNANT MENINGIOMAS

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INTRODUCTION: Invasive and malignant meningiomas present a significant therapeutic challenge due to high recurrence rates and invasion into surrounding bone, brain, neural and soft tissues. Understanding the mechanism of invasion could help in designing novel therapeutic approaches in order to prevent the need for repeat surgery, decrease morbidity and improve patient survival. The aim of this study was to identify the differential gene and protein expression profile in bone-invasive and malignant versus non-invasive meningiomas, focusing on factors that regulate invasion and identifying molecular mechanism of invasion. METHODS: Samples from bone-invasive, non-invasive and malignant meningiomas were used for RNA microarray, quantitative real-time PCR and Western blot analyses. Malignant meningioma cell lines (F5) were used for in vitro and in vivo functional assays. RESULTS: RNA microarray data analysis identified over 300 differentially expressed genes in bone-invasive versus non-invasive meningiomas. Ingenuity pathway analysis showed cell movement and invasion pathway among the significantly enriched networks, with an increased expression of its molecules including ADAMTS4, MMP19 and MMP16. Increased expression of these molecules was also identified in malignant meningiomas. Among these proteins, only MMP16 was identified as a secreted form in the condition media of the F5 cells. Knock down of MMP16 resulted in reduced MMP9 and MMP3 activity as determined by zymography and decreased invasion and migration of tumor cells both in vitro and in vivo. CONCLUSION: Our data identifies MMP16 as a novel factor involved in regulating the invasive phenotype in meningiomas and modulating MMP16 alters invasion and overall growth potential of intracranial meningioma models. Therefore, MMP16 can be considered as a potential therapeutic target which we are exploring in future studies with the aim to translate to clinical studies.

CB-034. HYPOXIA INDUCED ROS PRODUCTION REGULATES STAT3 ACTIVATION FOLLOWED BY ANGIOGENESIS IN HUMAN GLIOBLASTOMA

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OBJECTIVE: Glioblastoma has typical histopathologic findings, pseudopalisading necrosis and microvascular proliferation, all of which are associated with hypoxia. In a previous report, we found that Stat3 plays an important role in glioblastoma angiogenesis and migration by hypoxia. However, it is poorly understood for underlying mechanism of Stat3 activation in hypoxic condition. In this study, we examined reactive oxygen species (ROS) for hypoxia-induced Stat3 activation followed by angiogenesis in human glioblastoma. METHODS: To determine reactive oxygen species (ROS) production in hypoxic human glioblastoma cells, we examined ROS by flowcytometry in 1% O_2 condition. By using western blot, we examined the effect of antioxidant drugs on Stat3 activation in hypoxic condition. In addition, we determined the origin of ROS by DCF-DA, Mitosox by immunocytochemistry and NOX expression by real time RT-PCR and western blot. Furthermore, we examined suppressive effect of NOX on the Stat3 expression by RNA interference system. Finally, we examined ROS induced VEGF expression and angiogenesis by ELISA and tube formation assay in hypoxia. RESULTS: In 1% O2 condition, ROS was increased in glioblastoma cells compared to normoxic ones. By using antioxidant treatments such as DPI, NAC, Stat3 activation was decreased in hypoxic glioblastoma. For the intracellular localization, ROS was not only increased in the mitochondria but in the cytoplasm by DCF-DA and Mitosox immunostaining. In addition, NOX was increased in hypoxic condition and NOX4 inhibition by siRNA suppressed ROS and Stat3 activation. Finally, ROS inhibition decreased VEGF expression and tube formation in hypoxic glioblastoma. CONCLUSION: Hypoxia induced ROS production may regulate Stat3 activation followed by angiogenesis in glioblastoma. Considering that the hypoxia is associated with poor prognosis, inhibition of ROS induced Stat3 activation can be a new therapeutic target for human glioblastoma and induce longer survival.

CB-035. HOMEOBOX GENE HOXA10 IS RELATED WITH TEMOZOLOMIDE RESISTANCE BY REGULATING HOMOLOGOUS RECOMBINANT DNA REPAIR PATHWAY IN GLIOBLASTOMA CELL LINES

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O^6-methylguanine DNA methyltransferase (MGMT), mismatch repair (MMR), and homologous recombinant (HR) pathways are three DNA repair pathways involved in anticancer mechanism of temozolomide. We focused on HR pathway, which is relatively less investigated for temozolomide resistance, and homeobox gene HOXA10 for its candidate regulator. Homeobox genes play essential role in embryonic development, but are known to be aberrantly expressed in glioblastomas. In this study, we used two glioblastoma cell lines with different MGMT status; LN18 (unmethylated MGMT promoter) and LN229 (methylated MGMT promoter). Synergistic anticancer effect of HOXA10 inhibition with temozolomide was observed regardless of MGMT status. We found that HOXA10 inhibition is related with impaired double strand DNA breakage repair and decreased expression of Rad51 genes. Screening of differential gene expression between cell lines with or without HOXA10 inhibition using mRNA microarray and further

assessment revealed that early growth response 1 (EGR1) gene and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) are the mediator in the HR pathway regulation by HOXA10. Moreover, HOXA10 inhibition selectively affected Rad51 gene transcriptional regulation function of PTEN without interfering phosphatidylinositol 3-kinase (PI3K)/Akt-1 signaling pathway. In conclusion, our results shows that mechanism of HR pathway regulation by HOXA10 can be a possible explanation for the temozolomide non-responders of MGMT-inactive GBM and suggests that regulation of HOXA10 harbours an another target mechanism for overcoming temozolomide resistance in glioblastoma patients.

CB-036. S-NITROSYLATION OF THE p53 TUMOR SUPPRESSOR PROTEIN IN MEDULLOBLASTOMA

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p53 dysfunction plays a major role in the pathobiology of malignant brain tumors, and numerous post-translational modifications can regulate p53 activity. Nitric oxide, a gaseous signaling molecule, contributes to both physiologic and pathologic states through S-nitrosylation of protein cysteine residues, thereby altering protein function. Signaling associated with nitric oxide has increasingly been implicated in the biology of malignant brain tumors, and so we hypothesized that p53 might be regulated by S-nitrosylation. p53 contains ten cysteine residues, several of which are essential for zinc coordination and DNA binding. Using the biotin switch method, we detect S-nitrosylation of p53 in cells exposed to nitric oxide donors, as well as under physiologic conditions in mouse brain. We demonstrate p53 S-nitrosylation in ONS76 medulloblastoma cells which express endogenous neuronal nitric oxide synthase (nNOS). Manipulation of nitric oxide levels in cell lines suggests nitric oxide-dependent regulation of p53-mediated transcriptional activity and cell death. S-nitrosylation is a novel post-translational modification of p53 that may regulate p53 response to cell stressors and contribute to the pathogenesis of medulloblastoma and other malignant brain tumors in which nitric oxide is generated.

CB-037. GENE-EXPRESSION PROFILING ELUCIDATES MOLECULAR SIGNALING NETWORKS THAT CAN BE THERAPEUTICALLY TARGETED IN VESTIBULAR SCHWANNOMA

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Vestibular schwannomas (VS) are common benign tumors of the vestibular nerve that cause significant morbidity. The current treatment options for VS include surgery or radiation with each treatment option having associated complications and morbidities. The transciptome of schwannoma is still largely unknown. In this study we performed gene expression profiling of 49 schwannomas and 7 normal control vestibular nerves to identify differentially expressed genes. We identified over 4000 differentially expressed genes between control and schwannoma with network analysis uncovering proliferation and anti-apoptotic pathways previously not implicated in VS. Furthermore, using several distinct clustering technologies, we could not reproducibly identify subtypes of schwannomas suggesting that our schwannoma cohort was molecularly distinct from normal tissue yet highly similar amongst themselves. At the molecular level the PI3K/AKT/mTOR signaling network was overexpressed in our schwannoma cohort and evaluated for therapeutic targeting. Testing compounds BEZ235 and PKI-587 both novel dual inhibitors of PI3K and mTOR attenuated tumour growth in a preclinical cell line model of schwannoma (HEI-293). In vitro findings demonstrated that ablation of the PI3K/AKT/mTOR pathway with next generation inhibitors lead to decreased cell viability and increased cell death. Elucidation of novel molecular targets in vestibular schwannoma by transcriptional profiling versus appropriate controls may lead to promising effective therapeutic strategies and shed insight into the molecular ontogeny of this tumour.

CB-038. ERLOTINIB RESISTANCE IN EGFR-AMPLIFIED GLIOBLASTOMA CELLS IS MEDIATED BY UP-REGULATION OF EGFRvIII AND PI3K p110δ

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BACKGROUND: Treatment efficacy of EGFR tyrosine-kinase inhibitors like Erlotinib has not met expectations for glioblastoma therapy in clinical trials, even for EGFR-overexpressing tumors. We determined possible mechanisms of therapy resistance using the unique BS153 glioblastoma cell line that has retained amplification of the egfr gene and expression of the constitutively active receptor variant EGFRvIII. METHODS: Functional effects of Erlotinib, Gefitinib and Cetuximab on BS153 proliferation, migration and EGFR-dependent signal transduction were systematically compared in vitro. Tumor-initiating capacity of parental and treatment-resistant BS153 was studied in NMRI/Foxn1nu mice. Potential mediators of resistance were knocked down using siRNA. RESULTS: Erlotinib and Gefitinib inhibited proliferation and migration of BS153 in a dose-dependent manner, whereas Cetuximab had no effect. BS153 developed resistance to Erlotinib (BS153resE) but not to Gefitinib. Resistant cells displayed significantly decreased phosphorylation of all major receptor tyrosine kinases (Met, PDGFRα, HER2) except for EGFR. Resistance was associated with strong upregulation of EGFRvIII and subsequent activation of the phosphatidylinositol-3-OH kinase (PI3K)-pathway in BS153resE and an increased expression of the regulatory 110kDa delta subunit of PI3K (p110δ). Knockdown of EGFRvIII in BS153resE largely restored sensitivity to Erlotinib. Targeting PI3K pharmacologically caused a significant decrease in cell viability, and specifically targeting p110δ by siRNA partially restored Erlotinib-sensitivity in BS153resE. In vivo, BS153 formed highly invasive tumors with an unusual growth pattern, displaying numerous satellites distant from the initial injection site. Erlotinib resistance led to delayed onset of tumor growth as well as prolonged overall survival of mice without changing tumor morphology. CONCLUSION: EGFRvIII can mediate resistance to Erlotinib in EGFR-amplified glioblastoma via an increase in PI3Kp110δ. Interfering with PI3Kp110δ can restore sensitivity towards the TKI.

CB-039. NUCLEAR FACTOR I A (NFIA) NEGATIVELY REGULATES p53, p21, AND PAI1 TO PROMOTE THE MALIGNANT BEHAVIOR OF GLIOBLASTOMAS

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Glioblastoma (GBM) represents the most common primary malignant brain cancer and carries a dismal prognosis. Recent evidence showed that Nuclear Factor I A (NFIA), a transcription factor and essential regulator in embryonic glial development, is highly expressed in human GBM compared to normal brain, suggesting that NFIA may have a role in astrocytoma biology. However, the contribution of NFIA to GBM pathogenesis remained unknown. Here, we show that NFIA promotes growth and migration of GBM and establish the molecular mechanisms mediating these functions. NFIA overexpression accelerated growth, proliferation and migration of GBM in cell culture and in mouse brains whereas knockdown of native NFIA blocked tumor growth and induced cell death and apoptosis. These NFIA tumor-promoting effects were mediated by transcriptional repression of p53, p21, and plasminogen activator inhibitor 1 (PAI1) through specific NFIA-recognition sequences in their promoters. Importantly for GBM, in which TP53 is frequently mutated, the effects of NFIA on proliferation and apoptosis were independent of TP53 mutation status. Thus, NFIA is a previously unrecognized modulator of GBM growth and migration, which functions by distinct regulation of critical oncogenic pathways that govern the malignant behavior of GBM.

CB-040. Oct 7 IS EXPRESSED IN HUMAN GLIOMAS AND CORRELATES WITH MALIGNANCY GRADE

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INTRODUCTION: The transcription factor Oct7 (also called POU3f2/Brn2) is expressed during neurogenesis and constitutes the CNS equivalent of Oct 4, a critical regulator of induced pluripotent stem cells. Moreover, data suggest its expression is regulated by hypoxia, and Oct7 expression has been reported in malignant melanoma of the skin. Since both melanomas and CNS-malignancies arise in organs of neuroectodermal origin, we investigated whether human gliomas expressed Oct7. MATERIAL AND METHODS: We performed immunohistochemistry of 150 grade II-IV gliomas from our tumor bank, and subsequently performed western blots of 20 glioma samples. In addition, we performed flow cytometry analysis of Oct7 expression in 5 acutely dissociated tumors. Using lentiviral transfection we established Oct7 overexpression in a panel of constitutively negative glioma cell lines, as well as knock-down in an Oct7 positive glioma cell line, to investigate the effect of Oct7 expression on proliferation, migration and differentiation. RESULTS: Immunohistochemistry showed that Oct7 was almost uniformly expressed in human gliomas, although at a varying degree. Microscopy revealed a predominantly nuclear staining pattern, but cytoplasmic immunopositivity for Oct7 could also be detected in some tumors. Both western blot and flow cytometry confirmed expression of Oct7 in human gliomas. With two independent observers we obtained a nuclear staining index for all tumors, with 61% positive nuclei in GBM specimens, which was significantly higher than for grade II (37%) and grade III (36%) tumors ($p = 0.0001$). Moreover, ongoing studies suggest that overexpression of Oct7 increases the proportion of cells in G2/M phase of the cell cycle, suggesting that this transcription factor has a role in regulating tumor cell proliferation, and hence possibly overall tumor aggressiveness. Ongoing studies aim at further elucidating the multiple roles of Oct7 in brain tumor progression.

CB-041. PATTERNS OF MOLECULAR HETEROGENEITY IN RODENT MODELS OF GLIOBLASTOMA

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INTRODUCTION: Gliomas are the most malignant brain tumors with a dismal survival rate and no effective treatment. Recruitment of glial progenitors by platelet-derived growth factor (PDGF) signaling has been implicated in the degree of proliferation and invasion in the proneural phenotype of glioma. Various mathematical models have correlated levels of PDGF to recruitment of healthy oligodendrocyte progenitor cells (OPC's) into the tumor. We sought to determine whether PDGF is uniformly distributed through the tumor or whether it is expressed heterogeneously within the tumor, and to correlate those levels with changes in global gene expression in heterogeneous regions of the tumor. METHODS: By stereotactically injecting PDGF overexpressing retrovirus into subcortical white matter of rodent brains we generated tumors with the histological and molecular hallmarks of proneural glioblastoma. After isolating coronal sections harboring the tumor focal core biopsies were taken from the center of the tumor continuously to the invasive border and beyond, as well as in a grid pattern encompassing the entire tumor and peripheral tissue. Using sandwich ELISA, the PDGF levels within each biopsy were measured. Subsequently, high throughput RNA sequencing was employed to quantify global gene expression within regions of the tumor. RESULTS: PDGF concentrations at the core of the tumor exist at strikingly high concentrations that are well beyond physiological saturation. Concentration of PDGF declines sharply to undetectable levels in adjacent biopsies, and global expression patterns show high degree of heterogeneity. CONCLUSION: Our results are consistent with the established histological heterogeneity of glioblastoma, which may in part, be due to a heterogeneous molecular microenvironment. Furthermore, findings of molecular heterogeneity can guide the treatment of glioblastoma in the clinical setting.

CB-042. UP-REGULATION OF THE CHAPERONE PROTEIN PROLYL 4-HYDROXYLASE, BETA POLYPEPTIDE (P4HB), PROMOTES TUMOUR INVASION, ANGIOGENESIS AND GROWTH VIA EGFR/MAPK (ERK) SIGNALING

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INTRODUCTION: Endoplasmic reticulum (ER) chaperones have received considerable attention as emerging therapeutic targets. We have recently demonstrated that dysregulation of one of the chaperones, prolyl 4-hydroxylase, beta polypeptide (P4HB), may play a role in determining chemodrug sensitivity through the unfolded protein response (UPR) in glioblastoma multiforme (GBM). OBJECTIVE: To investigate the role of P4HB in gliomagenesis. The hypothesis was that P4HB could promote tumour growth and invasion in glioma. MATERIALS AND METHODS: We examined correlations between P4HB expression with clinical and pathological parameters using clinical specimens and publicly available database (GEO). Gene clustering and pathway analysis were performed using GSE16011 database. Cell proliferation and invasion were assessed by in vitro assays using GBM cells with knockdown (D54- and U87-shP4HB) and forced expressed P4HB (U87- and U251-P4HB). Western blotting was employed to confirm phenotypes and activation of signaling pathways. We examined in vivo tumour growth by bioluminescence imaging of luciferase-tagged (U87- and U251-VEC); (U87- and U251-P4HB) GBM cells in an orthotopic nude mouse model. H&E and IHC staining of invasive and angiogenic markers were performed on ex-vivo xenografts. RESULTS: High-grade gliomas (grade III and IV) had significantly higher P4HB expressions than low-grade gliomas and control brain tissues. High P4HB expression was significantly associated with upregulated expressions of both invasive (vimentin, MMP2 and MMP14) and angiogenic (CD31 and VEGF-A) markers. Pathway scan showed that P4HB was involved in the activation of EGFR/MAPK (ERK) signaling. GBM cells with enhanced P4HB expression exhibited a greater ability to proliferate and invade compared to the parental cells and cells with shP4HB. Moreover, U87- and U251-P4HB cell implants showed greater tumourigenicity in vivo. CONCLUSION: P4HB promotes tumour invasion, angiogenesis and growth via EGFR/MAPK (ERK) signaling. Targeting P4HB may serve as a novel approach in retarding critical pathways in the treatment of GBM.

CB-043. THE MAJOR VAULT PROTEIN MEDIATES SURVIVAL AND MIGRATION COMPETENCE OF HUMAN GLIOBLASTOMA CELLS VIA STABILIZATION OF THE EGFR/PI3K SIGNALING

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Although vaults are ubiquitously expressed and highly conserved ribonucleoparticles, their precise cellular functions are still enigmatic. Vaults are predominantly built of multiple copies of the major vault protein (MVP) shown to be upregulated during glioma progression and drug resistance development. Such we demonstrated that human astrocytic brain tumors including glioblastoma are generally high in vault levels while the normal brain is widely devoid of MVP. Consequently, the aim of our study was to investigate whether MVP itself has a pro-tumorigenic function and how it supports glioblastoma aggressiveness. Based on a large tissue collection we reconfirm MVP overexpression in gliomas as compared to healthy brain. The impact of vaults on human glioblastoma cell survival and migration competence was further analyzed using siRNA knock-down and dominant-negative genetic approaches. Finally, the role of MVP promoting subcutaneous and orthotopic tumor growth in SCID mice was tested. Our results demonstrate that MVP/vaults significantly support glioblastoma cell migration and invasion as well as starvation resistance. The enhanced aggressiveness was based on MVP-mediated stabilization of the epidermal growth factor receptor (EGFR)/phosphatidyl-inositol-3-kinase (PI3K) signaling axis supported by PTEN hyper-phosphorylation and translocation into the nucleus. Accordingly, MVP overexpression led to enhanced tumor growth and brain invasion in mouse subcutaneous and orthotopic xenograft models. Overall our data elucidate for the first time a direct tumor-promoting function of vaults based on EGFR/PI3K-pathway stabilization in human glioblastoma.

CB-044. REGULATION OF PRIMARY HUMAN ASTROGLIAL (HAG) CELL PROLIFERATION VIA A BRAIN-ENRICHED, NF-κB-SENSITIVE micro-RNA-125b (miRNA-125b)

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Micro RNAs (miRNAs) constitute a class of small, single-stranded, non-coding RNAs (sncRNAs) that bind, via base-pair complementarity, to the 3′

un-translated region (3′-UTR) of their target messenger RNAs (mRNAs), to ultimately down-regulate the expression of that mRNA target. RT-PCR-, LED-Northern- and miRNA array-based analyses have indicated that of the total number of known human miRNAs (currently about 2000), only about ~20 miRNAs are in high abundance in the human brain neocortex. This brain-enriched group includes a family of 5 inducible, NF-kB sensitive miRNAs whose members include miRNA-9, miRNA-34a, miRNA-125b, miRNA-146a and miRNA-155. Several of these inducible miRNAs appear to be involved in the regulation of cell cycling and astroglial cell proliferation. For example, it was found that levels of miRNA-125b were significantly up-regulated (a) in rapidly dividing human astroglial (HAG) cells in primary culture; (b) in human glioma and glioblastoma tissues obtained at biopsy; (c) in cultured human glioma and glioblastoma cell lines; and (d) in cytokine-stressed HAG cells. Interestingly, miRNA-125b, when added to cultured HAG cells induced astroglial cell proliferation, while increasing cytoplasmic-to-nuclear ratios and promoting cytoskeletal disruption and morphological distortion. We further demonstrate a strong positive correlation between up-regulated miRNA-125b and the astroglial cell-specific cytoskeletal cell markers glial fibrillary acidic protein (GFAP) and the type III intermediate filament protein vimentin. Both anti-NF-kB reagents (PDTC, CAY10512, and/or CAPE) and anti-miRNA-125b (AM-125b) strategies were found to down-regulate miRNA-125b and strongly inhibited astroglial cell proliferation. These results suggest that miRNA-125b contributes to both altered cytoskeletal morphology and astrogliosis, and that anti-miRNA-125b and/or anti-NF-kB-based strategies may be clinically useful in the treatment of neurological disorders involving astroglial cell proliferation. Support: NIH NIA AG18031; NIH NIA AG038834 (WJL)

CB-045. SUBTYPE-SPECIFIC DIFFERENCES IN THE COAGULOME OF GLIOBLASTOMA SUGGEST A LINK BETWEEN EGFR, TISSUE FACTOR AND REGULATION OF TUMOR ANGIOGENESIS, INFLAMMATION AND DORMANCY

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INTRODUCTION: Glioblastoma (GBM) is associated with florid angiogenesis, recruitment of inflammatory cells and pervasive intratumoral and peripheral thrombosis, features that are both pathogenically and diagnostically important. Our earlier studies revealed the genetic basis of these effects in that oncogenic epidermal growth factor receptor (EGFRvIII) was found to impact the expression of tissue factor (TF), coagulation factor VII (FVII) and protease activated receptors 1 and 2 (PAR-1/2) in human GBM cells. Here we extend this study by interrogating the coagulome of recently characterized molecular subtypes of GBM (Classical, Proneural, Neural and Mesenchymal) and by testing the functional role of TF in glioma progression. METHODS: Coagulome data were extracted from the publically available repository The Cancer Genome Atlas (TCGA), including 202 patients. Transplantable human and spontaneous mouse GBM models were used to test the effects of TF on disease progression. RESULTS: We observed striking differences and subtype-related gene expression patterns within the GBM coagulome, including a close parallel between high levels of EGFR, and dramatic elevation of TF in the classical GBM subtype. Loss of function experiments illustrated the contribution of TF to EGFRvIII-driven growth of GBM xenografts in mice. Notably, indolent and TF-negative human glioma cells (U373) remained viable, but dormant for over 250 days post subcutaneous and orthotopic inoculation, while the enforced expression of TF (TF-U373) in these cells was sufficient to trigger overt tumor formation. This was preceded by a latent phase during which glioma cells recruited CD11b+ myeloid cells and CD105+ blood vessels, and underwent mutational changes in a manner dependent on their TF status. CONCLUSION: GBMs trigger subtype/pathway-specific mechanisms of activated coagulation, of which only one is associated with the EGFR/TF axis. The continuum of TF-induced procoagulant, inflammatory and angiogenic responses contributes to tumourigenesis, including through mutational changes in indolent glioma cells.

CB-046. EVALUATION OF A NOVEL APPROACH TO CONSTRUCT A THREE-DIMENSIONAL (3D), ALL HUMAN IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER (B-BB) FOR BRAIN TUMOUR THERAPY

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The blood-brain barrier (B-BB) is a functional unit that consists mainly of endothelial cells (ECs) with specialised tight junctions (TJs), astrocytic endfeet, pericytes embedded in a basement membrane (BM) between the ECs and the astrocytes, and specific extracellular matrix molecules (ECM). Unfortunately, many studies involved with testing the ability of therapeutics to cross the B-BB use single monolayer cultures and rely on Trans-Endothelial Electrical Resistance (TEER) measurements without verification of endothelial tight junction formation. Here, we report the correlation between TEER values and endothelial tight junction expression (ZO-1 and occludin) in a 3D 'in vitro' model of the blood-brain barrier from human brain-derived cells and aim to create a model that more closely reflects the in vivo microenvironment. A 3D model of the B-BB was constructed using human brain endothelial cells (hCMEC/D3), human cortical astrocytes (SC-1800) and human brain vascular pericytes (HBVP) in mono-, co-, tri- cultures. TEER values were measured using a cell monitoring system- cellZscope®. TJ protein expression levels were examined by Western blotting and immunocytochemistry. Co-cultures and tri-cultures resulted in significantly higher TEER values compared to endothelial cells alone ($p < 0.05$). TEER values for co-culture of ECs and astrocytes were 4620 Ω/cm^2, and for tri-cultivation including pericytes, 4141.50 Ω/cm^2 compared to single EC cultures (2244 Ω/cm^2). Western blot analysis revealed higher expression of ZO-1 and occludin in co- and tri-culture conditions. ICC studies revealed localised expression at cell-cell contacts whereas in single cultures, fainter and more diffuse staining was observed. This study suggests that astrocytes contribute to form a tighter barrier in combination with endothelial cells. Whether perictyes influence the barrier formation or tightness of the B-BB remains to be elucidated. We are now designing targeted nanoparticles and assessing their ability to cross the B-BB using this 3D in vitro model.

CB-047. INFLUENCES OF DIFFERENT BLOOD-BRAIN BARRIER (B-BB) BASEMENT MEMBRANE ECM MOLECULES ON BRAIN TRANS-ENDOTHELIAL ELECTRICAL RESISTANCE

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The blood-brain barrier (B-BB) consists of endothelial cells (ECs) with specialised tight junctions (TJs), astrocytes, pericytes and specific ECM molecules. While much is known concerning the cells that make up the B-BB and the important role of each to B-BB function, little is recognised in regard to the contribution of the extracellular matrix (ECM) proteins that make up the basement membranes (BM) on B-BB function and integrity. This study aims to determine the effects of BM-ECM components on endothelial cell proliferation, adhesion and endothelial cell electrical resistance (Trans-Endothelial Electrical Resistance-TEER) to develop an in vitro model that better reflects the in vivo microenvironment. Human brain cerebral microvascular endothelial cells (hCMEC/D3) were used the present study. Cell seeding number was optimized for TEER values and then plated on different ECM molecules such as laminin (25-75 μg/ml), fibronectin (1-5 μg/ml), collagen type IV (7-10 μg/ml), agrin (1-3.5 μg/ml), and perlecan (2.5-10 μg/ml) under increasing concentrations and assessed for proliferation, adhesion and TEER changes using an Electric Cell-Substrate Impedance Sensing (ECIS) system. The individual optimal ECM concentrations for the highest and longest TEER values differed depending on ECM molecule and concentration. Cells plated on fibronectin and collagen type IV demonstrated highest TEER values compared to others. Interestingly, agrin at only 1 μg/ml had a significant effect on endothelial cell TEER values. Cell adhesion (%) correlated with TEER values obtained. Our preliminary results demonstrate that different B-BB basement membrane molecules have varied and profound effects on TEER values, an indicator of B-BB function. In addition, information concerning agrin and perlecan and their influence on brain endothelial cells in this context is novel. We are now looking at the effects of different ECM molecules on astrocytes and pericytes.

CB-048. YES-ASSOCIATED PROTEIN: A MASTER METABOLIC REGULATOR IN MEDULLOBLASTOMA

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Downstream of mitogenic Sonic hedgehog signaling, Yes-Associated Protein (YAP) can drive proliferation in Cerebellar Granule Neural Progenitor cells, and its ectopic expression promotes highly aggressive Shh-driven medulloblastoma growth and radio-resistance (Fernandez et al., 2009). More recently we have found that YAP can regulate Fatty Acid Synthesis (FAS) enzymes in CGNPs, independent of Sonic Hedgehog (Shh). Deregulating the metabolic machinery for aberrant energy utilization is one of the hallmarks of a proliferating cancer cell. To gain further insight into lipid regulation in Shh medulloblastomas, we carried out lipid mass spectrometry, and we found that Shh mouse medulloblastomas feature high levels of

cholesteryl ester accumulation. Cholesteryl ester synthesis from free cholesterol is catalyzed by the enzyme Sterol O-acyltransferase 1 (SOAT1). To determine whether YAP regulates SOAT and other metabolic enzymes, we performed a co-immunoprecipitaton of YAP followed by mass spectrometry and analysed the results with Ingenuity Pathway Analysis (IPA). We found that predicted direct and indirect targets of YAP comprised multiple metabolic pathways, including cholesteryl esterification and FAS, consistent with our experimental results. The present study has attempted to dissect the mechanism by which YAP regulates SOAT expression and activity and also confirm the IPA-predicted metabolic targets of YAP. The result of disrupting these pathways through YAP ablation and pharmacological means, on CGNP and medulloblastoma cell proliferation, is also presented. The study investigates YAP-regulated metabolic pathways as potential targets for novel medulloblastoma therapies that may reduce or eliminate the requirement for high dose radiation.

CB-049. HCMV GLYCOPROTEIN B IS EXPRESSED IN PRIMARY HUMAN GLIOBLASTOMAS AND ENHANCES GROWTH AND INVASIVENESS VIA PDGFRα ACTIVATION

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Our laboratory was the first to demonstrate that the ubiquitous human pathogen cytomegalovirus (HCMV) is highly associated with the most common and deadly form of primary brain tumor, glioblastoma (GBM). We believe that HCMV can act as an oncomodulator of glioblastoma by driving cellular signaling pathways essential to the neoplastic process. Our previous work demonstrated that the viral surface glycoprotein B (gB) mediates viral attachment, penetration, and cell-to-cell spread via the cellular receptor tyrosine kinase PDGFRα which results in activation of the PI3K/Akt pathway. Because this signaling pathway is implicated in gliomagenesis, we hypothesized that persistent expression of gB in tumor cells could enhance glioma cell invasiveness and growth by activating PDGFRα and downstream oncogenic pathways that regulate cells survival and invasion. Using RT-PCR, immunofluorescence, and western blot approaches we identified gB expression in several primary GBM tissue samples and demonstrated that expression of gB in glioma cells resulted in constitutive phosphorylation of PDGFRα, Akt, Src, and FAK. Incubation of U87 or primary GBM cells with recombinant gB or whole virus resulted in increased migration in transwell, Matrigel and brain slice invasion assays, which was specifically inhibited with neutralizing antibodies to gB or PDGFRα. Likewise, infection of U87 cells with HCMV stimulated motility in a wound healing scratch assay in a PDGFRα and integrin αvβ3-dependent manner. Stable expression of gB in U87cells enhanced in vitro migration and proliferation as well as in vivo tumor growth in an intracranial xenograft mouse model. Immunohistochemical staining of xenograft tissue sections demonstrated sustained expression of gB in vivo with an associated increase in phosphor-Akt and tumor cell dispersal relative to controls. Our results suggest that HCMV gB can induce key hallmarks of glioma, i.e., tumor cell proliferation and invasiveness which could lead to a more aggressive glioblastoma phenotype.

CB-050. NF-κB INDUCED IL-6 ENSURES STAT3 ACTIVATION AND TUMOR AGGRESSIVENESS IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most aggressive and neurologically destructive tumor of the central nervous system (CNS). In GBM, the transcription factors NF-κB and STAT3 are aberrantly activated and associated with tumor cell proliferation, survival, invasion and chemoresistance. In addition, common activators of NF-κB and STAT3, including TNF-α and IL-6, respectively, are abundantly expressed in GBM tumors. Herein, we sought to determine the signaling crosstalk that occurs between the NF-κB and STAT3 pathways in GBM tumors. We demonstrate that TNF-α-induced activation of NF-κB is sufficient to induce IL-6 expression, activate STAT3, and elevate STAT3 target gene expression in human and murine GBM cell lines as well as primary human GBM neurospheres *in vitro*. Using the clinically relevant human GBM xenograft model, we assessed the efficacy of inhibiting NF-κB and/or STAT3 alone or in combination in mice bearing intracranial xenograft tumors *in vivo*. We determined that the combined inhibition of NF-κB and STAT3 signaling significantly increases survival of mice bearing intracranial tumors when compared to single agent therapy. Furthermore, the NF-κB and STAT3 pathways contribute to cellular communication within the tumor environment, activating additional non-tumor cells that then suppress immune defense mechanisms and allow continued growth of the tumor. We are currently evaluating the effect of NF-κB and STAT3 inhibition on tumor infiltrating macrophages/microglia in a variety of orthotopic tumor models, including a syngeneic model which lacks SOCS3, the negative regulator of STAT3, in myeloid lineage cells. We believe that these studies will verify that pharmacological interventions to effectively inhibit the activity of both NF-κB and STAT3 will correlate with reduced GBM size and aggressiveness, and are imperative to more accurately develop pharmacological clinical interventions for patients with GBM tumors.

CB-051. INTER-CELLULAR SIGNALS WITHIN HETEROGENEOUS GBM CELLS INDUCE TUMOR CELL REPOPULATION AFTER RADIATION TREATMENT

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Glioblastoma multiforme (GBM) is the most frequent and lethal form of primary brain cancer. Radiation represents the most effective post-surgical therapy, however radiotherapy is only palliative and the mechanisms underlying tumour radioresistance remain elusive. Among heterogeneous GBM cells, glioma stem cells (GSCs) are defined as a subpopulation that is relatively resistant to radiotherapy compared to non-GSCs in tumors. Here we tested the hypothesis that radiation treatment for GBM activates inter-cellular signals between GSCs and non-GSCs to facilitate surviving GSCs' proliferation, thereby promoting tumor cell repopulation to gain therapy resistant phenotype. Our data demonstrated that the conditioned medium from radiated GBM cells increases the growth of GSCs in culture. Phenotypically, GSCs that were stimulated with the conditioned medium from radiated GBM cells shifted their phenotype from proneural to mesenchymal, suggesting that inter-cellular signals induces proneural-to-mesenchymal transition (PMT) of GSCs. Further, gene expression analysis of radiation treated GBM cells induces Wnt expression as well as the upregulation of the stress-induced kinase MELK. Given that we recently identified the tumor-specific protein complex of MELK with the oncogenic transcription factor c-JUN, these data may indicate that WNT signaling is a downstream target of MELK and c-JUN in GBM cells. Currently, ongoing experiments include the in vivo validation of the in vitro PMT of GSCs, characterization of signaling pathways to regulate PMT of GSCs, and identification of inhibitors for this transition. Together, we will seek to determine the inter-cellular signals between GSCs and non-GSCs to promote tumor evolution to acquire therapy resistant phenotype.

CB-052. JAK2-STAT3 ACTIVATION IS ASSOCIATED WITH CEREBROSPINAL FLUID INTERLEUKIN-10 (IL-10) IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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OBJECTIVE: The Janus kinase 2(JAK2)-signal transducers and activators of transcription 3(STAT3) signaling pathway is constitutively activated in various cancers. In the present study, we examined JAK2-STAT3 activation in primary central nervous system lymphoma (PCNSL) and analyzed a relationship between the activation of the JAK2-STAT3 pathway and the CSF levels of IL-10 and IL-6. METHODS: Thirty-five adult patients with PCNSL newly diagnosed from January 2004 to September 2012 were analyzed retrospectively. In all patients, IL-10 and IL-6 levels of CSF were measured preoperatively. In 35 patients, we examined activation levels of JAK2 and STAT3 in surgically obtained PCNSL tissues by immunohistochemistry. Relationship between JAK2 or STAT3 activation levels and CSF IL-10 or IL-6 levels was analysed. The pJAK2 and pSTAT3 expression was scored semi-quantitatively (score 1-6). STAT3 activation was also analyzed in 17 frozen samples by western blot and compared to the CSF IL-10 levels. RESULTS: The expression level of the pSTAT3 was correlated with the expression level of the pJAK2 (Pearson's correlation coefficient test; $p < 0.01$). CSF IL-10 level was statistically correlated with pJAK2 expression (Student's t-test; $p = 0.02$) and pSTAT3 expression (Student's t-test; $p = 0.027$) in the immunohistochemical examination. However, there was no association between the CSF IL-6 levels and the pJAK2 or pSTAT3 expression levels. Western blot examination revealed that pSTAT3 expression levels were relatively increased in the high CSF IL-10 group compared with the levels in the low CSF IL-10 group,

although STAT3 expression levels were not different between the both groups. CONCLUSIONS: These results suggest that autocrine/paracrine stimulation by IL-10 via CSF may activate the JAK/STAT3 pathway in PCNSLs. CSF IL-10 levels may be a predictive marker of STAT3 activation in PCNSL.

CB-053. GENE EXPRESSION PROFILES OF SUNITINIB-TREATED BUT NOT UNTREATED SHORT-TERM SERUM-FREE CULTURES PREDICT TREATMENT RESPONSE OF HIGH-GRADE GLIOMAS IN VITRO

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The increasing knowledge of the pathogenesis and progression of high-grade gliomas has led to the development of a novel group of therapeutics which includes small molecule kinase inhibitors. These agents directly interfere with growth factor receptor signaling pathways which are upregulated in brain tumors and are supposed to impact oncogenesis. Despite promising preclinical studies, results of pilot trials have been generally disappointing. Nevertheless, a subgroup of patients responds to therapy, often with long-term disease stabilization. The molecular mechanisms which would explain the heterogeneity of response have not been understood yet. Diagnostic strategies to identify those patients that will profit from a specific targeted therapy are urgently needed. The most common approach for biomarker identification is the retrospective supervised analysis contrasting the expression profiles of responders with those of non-responders. We here suggest a novel strategy for developing signatures predictive to drug response. We postulate that for certain therapeutic agents predictive gene expression patterns emerge only after tumor cells have been treated with the respective agent in vitro beforehand. As a proof-of-principal we analyzed the global gene expression profiles in 18 short-term serum-free cultures of high-grade gliomas before and after in vitro treatment with the tyrosine kinase inhibitor Sunitinib. Furthermore, we examined the modulation of mitogenic signaling after treatment and analyzed the impairment of proliferation and migration capacity in vitro. In every aspect, glioma cultures exhibited extremely individual responses. Signaling analysis by Western-blot did not predict in vitro response. However, genetic profiles evaluated by microarray from treated but not from untreated glioma cultures allowed to predict therapy induced impairment of proliferation in vitro. Prediction can be achieved with as little as 6 genes possibly allowing for a straightforward translation into the clinic once the predictive power of the signature is shown in vivo in the context of a clinical study.

CB-054. TUMOR SUPPRESSIVE ROLE OF DACH1 IN GLIOBLASTOMA STEM-LIKE CELL

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DACH1 gene encodes a chromatin-associated protein that associates with other DNA-binding transcription factors and is reported to regulate gene expression and cell fate determination during development. Decreased expression of *DACH1* is reported in several types of cancers, and loss of its expression is suspected to contribute to tumorigenesis. Recently by allelic DNA copy number analysis using single-nucleotide polymorphism genotyping array and mass spectrometry, we identified homozygous deletions in glioblastomas at chromosome 13q21, where DACH1 gene is located. In addition, we observed frequent promoter methylation of DACH1 gene, which potentially cause down-regulation of DACH1 gene expression. Therefore, we hypothesized that DACH1 has tumor suppressive activity in glioblastoma. Indeed, forced *DACH1* expression decreased cell proliferation of glioma cell lines *in vitro* and growth of engrafted tumors in mice. In glioblastoma stem-like cells cultured in serum-free NBE medium supplemented with EGF and bFGF, the ability of spheroid formation was markedly disrupted by increased *DACH1* expression. We also reported that fibroblast growth factor 2 (FGF2/bFGF) is transcriptionally repressed by DACH1, in these cell lines. When we xenografted spheroid-forming cells that expressed DACH1 into the mouse brain, tumor formation was considerably suppressed; however such suppression could be rescued by bFGF expression. From these results, we think that DACH1 suppress growth of glioblastoma stem-like cells through the regulation of bFGF-mediated cell proliferation mechanisms. Here we further analyzed the expression levels of DACH1 and bFGF in several glioblastomas stem-like cell lines established from surgical specimens and demonstrated their role for glioma development.

CB-055. A NEW ROLE FOR PKM2 AS A DIRECT REGULATOR OF THE CELL CYCLE MACHINERY CONTROLLING MITOTIC PROGRESSION

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PKM2 is a phosphotyrosine-binding glycolytic enzyme that alternatively functions as an activator of transcriptional programs that drive GBM cells into the cell cycle. We noted, however, that in multiple GBM cell lines, PKM2 knockdown resulted in an accumulation of cells with 4N DNA content, >2 centrosomes, and defects in entry into mitosis. These events were driven by increases in HuR and p27 phosphorylation, both of which led to an accumulation of p27 protein. Both HuR and p27 are phosphorylated by the cyclin B/cdk1 complex, which also controls entry of cells into mitosis. Accordingly, PKM2 knockdown decreased cdk1 activity while introduction of a constitutively active cdk1 reversed the effects of PKM2 knockdown on HuR, p27, and cell cycle progression. The means by which PKM2 increases cdk1 activity have not been described. In cycling cells, however, cdk activation is dependent on the transient interaction of T14/Y15-phosphorylated cdk1 with cyclin B, which allows for cdk7-mediated T161 cdk1 phosphorylation, cdc25C-mediated removal of pT14/Y15, and complex stabilization/activation. While PKM2 modulation did not influence cdk7 activity, forms of PKM2 that retained phosphotyrosine binding co-immunoprecipitated with pY15-containing cyclinB-cdk1 (but not with pY15 cdk1 monomers) and enhanced formation of active pT161 cdk1/cyclin B complexes. Furthermore, exogenous expression of only these forms of PKM2 reversed the effects of PKM2 knockdown on mitotic progression. These results show that PKM2 uses its ability to bind phosphotyrosine-containing proteins to stabilize otherwise transient pY15-containing cyclin B-cdk1 complexes. Complex stabilization in turn facilitates cyclinB-cdk1 activation, p27 suppression, and entry of cells into mitosis. These studies therefore define a new role for the metabolic enzyme PKM2, namely as a direct regulator of the cell cycle machinery that controls mitotic progression and the growth of brain tumor cells.

CB-056. DOWN-REGULATION OF MDR BY Ad-REIC CONTRIBUTES TO AUGMENT THE CHEMOTHERAPY BY TEMOZOLOMIDE

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BACKGROUND AND PURPOSES: Multidrug resistance (MDR) is a well-known drug efflux pump whose overexpression has been primarily observed in human cancer cells that are resistant to chemotherapy. MDR encodes for a P-glycoprotein and has been found to be expressed in majority of brain tumors, including glioblastomas. It has been reported that MDR activity was inhibited via JNK activation. We previously reported that the overexpression of REIC-Dkk3 exerts anti-tumor effects in a glioblastoma cell lines and it is associated with JNK activation. However, the relationship between REIC-DKK3 and MDR remains to be elucidated. In this study, we examined whether adenovirus vector REIC-Dkk3 (Ad-REIC) augments the anti-tumor effects of temozolomide by down-regulating MDR in glioblastoma. METHODS: We treated glioblastoma cells (U87MG) with temozolomide or Ad-REIC alone or in combination and compared to the cells treated with Ad-LacZ. The gene and/or protein expression of MDR, JNK, pJNK, c-JUN and p-c-JUN was analyzed by western blot, immunohistochemistry and quantitative RT-PCR. RESULTS: Effects of Ad-REIC or temozolomide alone on cell survival were limited in the U87MG cells. However, the combination of Ad-REIC and temozolomide induced significant cell death. In cells treated with Ad-REIC the expression of MDR1 was significantly reduced compared to cells treated with Ad-LacZ or temozolomide alone. Furthermore, Ad-REIC treatment increased JNK, pJNK, c-JUN and p-c-JUN levels. Applications of a JNK inhibitor reverse the effects of Ad-REIC on MDR

expression and cell death. These results suggest that Ad-REIC contributes to the improvement of chemosensitivity to temozolomide therapy by downregulation of MDR through activation of JNK. CONCLUSION: Ad-REIC gene therapy may be a potential therapy for glioblastoma.

CB-057. THE microRNA-31/TRADD/NF-κB CIRCUITRY IS DYSREGULATED IN GLIOBLASTOMA

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GBM are the most common and deadliest tumors of the CNS. Despite aggressive therapeutic approaches, these tumors remain incurable. The NF-κB family of transcription factors mediates immune and inflammatory signaling. Normally, NF-κB is inhibited by interactions with IκBα. However, in response to a stimulus such as TNFα, NF-κB becomes activated. Specifically, TNFα binds to its receptor, TNFR1, which promotes oligomerization and TRADD recruitment. Next, TRADD initiates a signaling cascade that ultimately activates NF-κB. Normally, the activity of NF-κB is tightly regulated. However, in many tumors, including GBM, NF-κB is constitutively activated and its target genes are overexpressed. MicroRNAs (miRs) are a class of short, endogenous, single-stranded RNA molecules that bind specific mRNA to inhibit their translation. Genes encoding miRs are often found in Cancer Associated Genomic Regions (CAGRs) and are usually dysregulated in cancer. This leads to disrupted mRNA and protein expression, and contributes to tumor development and/or progression. miR-31 is a microRNA with pleiotropic properties. In many cancers, miR-31 expression is reduced or absent. Herein, we demonstrate that homozygous deletion of miR-31 is most pronounced in GBM compared to other cancers analyzed, and its loss occurs in >35% of all GBMs. We find that miR-31 regulates TRADD expression and show that in response to TNFα stimulation, miR-31 is sequestered in the nucleus, thus stabilizing TRADD and allowing NF-κB activation. However once activated, NF-κB increases miR-31 levels, which reduce TRADD levels and limit NF-κB signaling. In vivo, the loss of miR-31 correlates with increased NF-κB activity and tumor size. Conversely, miR-31 restoration reduces NF-κB activity and limits tumor growth. Finally, in patients, the loss of miR-31 translates to significantly shorter survival rates. Collectively, these data underscore the important role of miR-31 in glioma biology, and highlight its attractive therapeutic potential in treating GBM.

CB-058. REGULATION OF CANCER PROMOTING TRYPTOPHAN CATABOLISM IN GLIOBLASTOMA BY THE GLUCOCORTICOID RECEPTOR

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The immunosuppressive microenvironment is a major obstacle for the treatment of glioblastoma. Recently, we identified a novel signaling pathway in glioblastoma and other types of cancer involving the degradation of tryptophan to kynurenine by tryptophan-2,3-dioxygenase (TDO), which leads to the inhibition of antitumor immune responses and promotion of tumor cell invasiveness. TDO was previously believed to be restricted to liver cells and responsible for maintaining systemic tryptophan homeostasis. Hence, nothing is known about the signaling pathways involved in the regulation of TDO in cancer. This study aimed at investigating the signals leading to the constitutive expression of TDO in glioblastomas. A siRNA-based transcription factor profiling revealed that the expression of human TDO is suppressed by endogenous glucocorticoid signaling. Similarly, treatment of glioblastoma cells with the synthetic glucocorticoid dexamethasone led to a reduction of TDO expression and activity. The glucocorticoid receptor (GR) mediated TDO inhibition was dependent on the immunophilin FKBP52, whose FK1 domain physically interacted with the GR as demonstrated *in vitro* by bimolecular fluorescence complementation. After knockdown of either GR or FKBP52 the dexamethasone mediated TDO downregulation was abolished. Most important, this interaction also takes place *in vivo* as demonstrated by *in situ* proximity ligation assay on human glioblastoma tissue. Accordingly, gene expression profile analyses revealed negative correlations of the GR and FKBP52 with TDO in glial and neural tumors. In summary, we identify a novel steroid-responsive FKBP52-dependent pathway suppressing the expression and activity of TDO, a central enzyme in human brain tumor immunobiology.

CB-059. Mir-128 CONTROLS THE ACTIVITY OF EPIGENETIC REGULATORS PRC1 AND PRC2 IN NEURAL STEM CELLS: IMPLICATIONS OF ITS LOSS IN GLIOMAGENESIS

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Polycomb Repressor Complexes (PRC) 1 and 2 are major chromatin modifiers whose coordinated activity determines histone modification of H2a and H3, respectively. This results in transcriptional repression of genes involved in cellular differentiation, maintaining cellular stemness. In glioblastoma, PRC activity has oncogenic functions and is involved in radio-resistance by favoring DNA Double Strand Break repair. While it is known that PRC1 and PRC2 are functionally related and partially redundant in their repressive action, little is known on the mechanisms that control their activity. Here we show that miR-128, downregulated in glioblastoma, directly targets BMI1 and SUZ12, key components of PRC1 and PRC2 respectively, thus exerting a fundamental role in PRC regulation and epigenetic gene control. The expression of mir-128 is inversely correlated to that of BMI1/SUZ12 in operative specimens of glioblastomas versus normal brain and in glioma stem cells versus neural stem cells. Gain of function of miR-128 results in decrease CD133 stem cell marker, reduced proliferation and impaired clonal ability of glioma initiating cells. Knock down of miR-128 in neural stem cells results in increase BMI1 and SUZ12 levels, associated to greater cell clonogenicity. In a genetic mouse model of glioblastoma, we found that miR-128 expression is blocked in young, pre-symptomatic mice, suggesting that loss of miR-128 expression is an early event in gliomagenesis. Furthermore, as BMI1 and SUZ12 take part in the cellular response to DNA double strand break repair, here we show that miR-128 prevents BMI1 and SUZ12 upregulation after irradiation resulting in an impaired capacity of DNA repair and consequent cell death. Finally, miR-128, although virtually undetectable, is not loss in glioblastoma initiating cells, and its expression can be stimulated by farmacologci treatment with cyclic AMP (cAMP). This is of great importance as it lays the bases for a technically achievable, miR-128-based therapy for glioblastoma multiforme.

CB-060. A KINOME-WIDE RNAi SCREEN IN DROSOPHILA GLIA REVEALS THAT THE RIO KINASES MEDIATE CELL PROLIFERATION AND SURVIVAL THROUGH TORC2-Akt SIGNALING IN GLIOBLASTOMA

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Glioblastomas frequently display mutations that activate receptor tyrosine kinase (RTK) and Pi-3 kinase (PI3K) signaling pathways. Yet, therapeutics targeting these pathways in glioblastomas have proven ineffective in the clinic. In Drosophila melanogaster, activation of RTK and PI3K pathways in glial progenitor cells creates malignant glial tumors that display many features of human glioblastoma. We used this Drosophila glioblastoma model to perform genetic screens for new genes required for RTK- and PI3K- dependent neoplastic transformation, and all of these genes were tested for tumor-specific functionality. Human orthologs of novel kinases with tumor-specific activities uncovered by these screens were functionally assessed in mammalian glioblastoma models and human tumors. From this screen, we isolated orthologs of the atypical RIO kinases, which, upon knockdown in Drosophila, caused a synthetic growth reduction and cell lethality in the context of oncogenic RTK-PI3K signaling. Our results from human glioblastoma cells and tumors revealed that RIOK1 and RIOK2 are overexpressed in tumor cells in response to Akt signaling downstream of oncogenic RTK-PI3K activity. Little to no RIOK1 or RIOK2 expression was detected in normal neurons and glia, suggesting that upregulation of RIO expression is tumor cell specific. Knockdown of

RIOK1 or RIOK2 in glioblastoma cells inhibited Akt-mTORC2 signaling and induced p53 activity, and caused cell cycle exit, apoptosis, and chemosensitivity. Conversely, overexpression of RIOK2 in immortalized astrocytes upregulated TORC2-Akt signaling and promoted tumorigenesis. Thus, RIO kinase activity was both necessary and sufficient to promote tumorigenesis. Moreover, our results imply that, in glioblastoma cells, constitutive Akt signaling drives RIO kinase overexpression, which creates a feedforward loop that promotes and maintains oncogenic Akt activity through stimulation of mTORC2 signaling. Thus, the RIO kinases may represent new therapeutic targets that disrupt tumor-specific TORC2-Akt signaling upon inhibition, which could prove useful for treatment of RTK- and PI3K- dependent glioblastoma and related cancers.

CB-061. AMP-KINASE ACTIVATION INDUCES RESISTANCE TO HYPOXIA AND TEMOZOLOMIDE IN GLIOBLASTOMA CELLS

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Glioblastomas (GB) are heterogeneous tumors. Their histological core features necrosis and neoangiogenesis mirror the nutrient-deprived conditions of the tumor-microenvironment. Standard therapy is palliative comprising surgery followed by radio- and chemotherapy, the latter most frequently using the alkylating agent temozolomide. Recently, antiangiogenic treatment with the VEGF-A antibody bevacizumab has been introduced for the treatment of GB. Chronic administration of bevacizumab causes pleiotropic effects including vascular regression and enhanced tumor hypoxia. AMP-Kinase (AMPK) is a major regulator of cellular energy homeostasis that signals to multiple targets including mTOR-complex 1 (mTORC1), a central nodal point in the regulation of cell growth and translation. New specific AMPK activators like A769662 have already been investigated for the treatment of metabolic disorders. In this experimental study, we found that A769662 profoundly protected human malignant glioma cells from glucose starvation as well as from hypoxia-induced cell death. Cells exposed to A769662 had reduced glucose consumption and decreased lactate production. Hypoxia alone caused an increase in reactive oxygen species (ROS). Surprisingly, A769662 also increased ROS levels under normoxic conditions and further increased ROS under hypoxia, although it conferred protection from hypoxia-induced cell death. On the other hand and in accordance with the elevated ROS levels, A769662 rendered cells more vulnerable to oxidative stress. Finally, A769662 inhibited mTORC1 signaling and reduced the sensitivity of glioma cells towards temozolomide. This is in line with our earlier finding that mTORC1 inhibition confers protection against other chemotherapeutic drugs including cisplatin and vincristine. Our results thus identify AMPK activation as a potential mediator of therapy resistance and a candidate target for inhibition.

CB-062. CARNOSINE AND ITS DERIVATIVES AS A PROSPECTIVE THERAPY FOR GLIOBLASTOMA MULTIFORME

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Carnosine (β-alanyl-L-histidine) is an endogenous dipeptide with high antioxidant and antiproliferative properties. The antioxidant properties of carnosine are evident from its ability to suppress lipid peroxidation, inhibit Fenton chemistry, and interact with superoxide. Recent evidence suggests that cellular reactive oxygen species (ROS; superoxide and hydrogen peroxide) regulate proliferation, and cellular ROS levels fluctuate through the cell cycle. Therefore, we hypothesize that the antiproliferative properties of carnosine are due to its ability to suppress cellular ROS levels and enhance cellular antioxidant capacity resulting in delays in cell cycle progression. Our results show that the antiproliferative effect of carnosine was more pronounced in human U-118-MG glioblastoma cells compared to MB-231 human mammary epithelial cancer cells, and Cal27 and FaDu human oral squamous cancer cells. This differential effect in cell doubling time correlated with a significantly lower level of carnosine synthase in U-118-MG cells. The dose dependent inhibition of cellular proliferation in carnosine treated U-118-MG cells was associated with a significant decrease in cellular ROS levels, assessed by flow cytometry measurements of DCFH oxidation. Carnosine-induced decrease in cellular ROS levels correlated with a significant increase in manganese superoxide dismutase (MnSOD) expression. Flow cytometry measurements of bromodeoxyuridine-positive (S-phase) and -negative (G_1 and G_2 phase) cells showed that carnosine treatments resulted in a significant increase in the percentage of G_2 cells, $37 \pm 0.4\%$ compared to $17 \pm 0.5\%$ in controls. Carnosine-induced G_2-accumulation was associated with 2.5- to 4-fold increase in cyclin B1 mRNA and protein levels. Among the carnosine derivatives tested, anserine (methylated carnosine) exhibited the most potent antiproliferative effect. These results demonstrate that the antiproliferative property of carnosine is due to its ability to enhance MnSOD expression and to induce G_2-delay. These results will be of significance in the potential clinical application of carnosine and its derivatives in glioblastoma therapy.

CB-063. GLIOMA-ASSOCIATED IDH MUTATION INDUCES miR-34a REPRESSION AND STEM CELL-LIKE PHYSIOLOGY THROUGH ENHANCED PDGF SIGNALING

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Point mutations in isocitrate dehydrogenase enzymes (IDH1 and IDH2) have been identified in 70-90% of lower-grade gliomas (LGGs; WHO grade II and III) as foundational genomic alterations. Glioma-associated IDH mutations occur in active site arginine residues and confer a neomorphic activity resulting in overproduction of the oncometabolite R(-)-2-hydroxyglutarate (2HG). The affects of 2HG on cellular physiology are widespread and include global shifts in epigenomic profiles. LGGs have also been repeatedly linked with dysregulated platelet-derived growth factor (PDGF) signaling. We have recently demonstrated that miR-34a is downregulated in gliomas in response to dysregulated PDGF signaling and that miR-34a directly targets the PDGF receptor (PDGFRA) in high-grade gliomas. Analyzing a panel of diffuse gliomas, we find that miR-34a is specifically downregulated in LGG in response to IDH mutation. Similarly, in multiple isogenic cell line systems, IDH mutation induces repression of miR-34a. Also in these contexts, IDH mutation induces neurosphere formation and the expression of stem cell-associated genes. miR-34a has been reported to target pluripotency factors such as LIN28, OCT4 and NANOG. Accordingly, we find that murine neuroglial progenitor cells expressing IDH mutant isoform express higher levels of these factors than isogenic controls. The stem cell-like phenotype of IDH mutant-expressing cells is morphologically reversed by restoring miR-34a expression in the cells. Despite findings that IDH mutation correlates with hypermethylation at the *MIR34a* locus in LGG tumors, we have not been able to demonstrate the functional relevance of this. Instead, we find that IDH mutation is associated with enhanced PDGF signaling, and we demonstrate that inhibition or stimulation of PDGF signaling in IDH mutant cells increases or decreases miR-34a expression, respectively. Our studies support a model in which glioma-associated IDH mutation induces miR-34a repression and stem cell-like physiology through enhanced PDGF signaling.

CB-064. PRONEURAL PHENOTYPE AND TRANSCRIPTIONAL NETWORK PRECEDES AND SELECTS FOR GENETIC ALTERATIONS DURING GLIOMA PROGRESSION

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Glioblastoma is a diverse disease that can be divided into distinct subtypes on the basis of molecular and genetic profiling. The Proneural subtype has an expression profile, which resembles that of oligodendrocyte progenitor cells (OPCs), and is associated with a specific set of genetic alterations. However the functional relationships between the Proneural phenotype and the associated genetic alterations have not been resolved. To address this issue, we used a mouse model of Proneural glioma and longitudinally tracked alterations in gene copy number (by array CGH) and expression profiles (by RNA-Seq) at multiple time points during tumor progression. We

showed that murine gliomas induced by retrovirus delivery of PDGF and Cre-mediated deletion of Pten, acquired a specific set of genetic deletions with remarkable consistency as they progressed from low to high grade tumors. Cross-species analyses revealed that subsets of these genes are also selectively deleted in human Proneural glioblastoma. Expression analysis at early time points showed that mouse tumors had a Proneural expression pattern, characterized by high levels of OPC genes, prior to the acquisition of the recurrent gene deletions. Further analysis identified transcription factors that act as master regulators of the Proneural phenotype, revealing a transcriptional network that was highly connected to p53 as one of the key master regulators at early and late stages of glioma progression. Experimental alteration of this regulatory network by upfront deletion of Trp53, led to accelerated glioma formation and obviated the acquisition of subsequent deletions. These results show the interplay between the pre-existing regulatory network, which defines cellular phenotype, and the genetic alterations that accumulate during progression of Proneural glioma.

CB-065. DIFFERENTIATION OF GLIOBLASTOMA MULTIFORME STEM-LIKE CELLS LEADS TO DOWN REGULATION OF EGFR AND EGFRvIII AND DECREASED TUMORIGENIC AND STEM-LIKE CELL POTENTIAL

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Glioblastoma multiforme (GBM) is the most common and devastating primary brain tumor among adults. Despite recent treatment progress, most patients succumb to their disease within 2 years from diagnosis. Current research have highlighted the importance of a subpopulation of cells, assigned brain cancer stem-like cells (bCSC), to play a pivotal role in GBM malignancy. bCSC are identified by their resemblance to normal neural stem cells (NSC), and it is speculated that the bCSC have to be targeted in order to improve treatment outcome for GBM patients. One hallmark of GBM is aberrant expression and activation of the epidermal growth factor receptor (EGFR) and expression of a deletion variant EGFRvIII. In the normal brain, EGFR is expressed in neurogenic areas where also NSC are located and it has been shown that EGFR is involved in regulation of NSC proliferation, migration and differentiation. This led us to speculate if EGFR and EGFRvIII are involved in the regulation of bCSC. In this study we use GBM neurosphere cultures, known to preserve bCSC features. We demonstrate that EGFR and EGFRvIII are down regulated upon differentiation and moreover that when EGFR signaling is abrogated, differentiation is induced. Furthermore, we show that differentiation leads to decreased tumorigenic and stem cell-like potential of the neurosphere cultures and that by specifically inhibiting EGFR signaling it is possible to target the bCSC population. Our results suggest that differentiation therapy, possibly along with anti-EGFR treatment would be a feasible treatment option for patients with GBM, by targeting the bCSC population.

CB-066. THE GLIAL DIFFERENTIATION FACTOR NUCLEAR FACTOR ONE B (NFIB) INDUCES DIFFERENTIATION AND INHIBITS GROWTH OF GLIOBLASTOMA.

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The phylogenetically-conserved vertebrate transcription factor, NFIB, is an orchestrator of glial differentiation in the developing mammalian central nervous system. We found NFIB expression to be reduced in glioblastoma (GBM), the commonest and most lethal primary adult brain cancer, so investigated what effect increased expression of NFIB had on GBM. Increased expression of NFIB in primary GBM cell lines induced expression of markers of glial differentiation, inhibited cell proliferation, reduced stem/progenitor cell growth, altered cell cycle progression and inhibited tumor growth in murine models of GBM. We thus identified NFIB to be a novel tumor suppressor gene in GBM.

CB-067. AUTOPHAGY BLOCKADE IN GBM IMPROVES SENSITIVITY TO TYROSINE KINASE INHIBITION

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Gliomas are the most common type of brain tumor and occur in both adults and children. Current treatments, radiation and cytotoxic chemotherapy (temozolomide), are ineffective, with an average survival of 14 months and a survival rate of approximately10%, indicating that new therapeutic agents need to be developed. The TAM family of receptor tyrosine kinases (RTKs), represented by Tyro-3, Axl, and MerTK, is ectopically expressed in glioma tumors, resulting in anti-apoptotic signaling, facilitating tumor cell survival. My preliminary data shows treatment of cells with radiation upregulates MerTK expression. Inhibition of TAM RTKs decreases cell survival, proliferation and migration. Previous studies in the lab have shown shTAM inhibition sensitizes cells to temozolomide. Radiation and temozolomide therapy increases autophagy, suggesting autophagy is a cytoprotective mechanism and therefore an attractive target. I hypothesize TAM inhibition and autophagy blockade will increase efficacy of glioma treatment. To conduct our experiments we utilized two primary patient glioblastoma cell lines and two commercially available glioblastoma cell lines. Using GFP-mCherry tagged LC3 protein along with western blot analysis we determined that inhibition of TAM, both genetically (shRNA) and pharmacologically (TKIs), leads to increased autophagic flux. Inhibition of autophagy flux, using chloroquine, in combination with TAM inhibition results in decreased proliferation compared to either agent alone. Additionally, we have confirmed that in our cell lines autophagy is increased post radiation treatment. These results suggest that TAM inhibition in combination with autophagy blockade results in more efficacious therapy for glioblastoma.

CB-068. BRAIN-SPECIFIC GENE SIGNATURE OF MELANOMA METASTASIS

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Melanoma brain metastases are highly resistant to therapy and carry a dismal prognosis. In this work, we sought to identify genetic drivers of melanoma brain metastasis using a reproductive and predictive xenograft model of human melanoma (Cancer Res 2013; 73; 2445–56). Immunodeficient mice were injected intracardially with a cell line developed in our laboratory from a resected tumor of a patient with multiple brain metastases. Bioluminescence imaging was used to follow the development of systemic metastases and MRI was used to track brain metastasis formation. The animals consistently developed metastases in their brains and frequently in the adrenals, ovaries, and femurs. These four organs were enzymatically dissociated using tailored-made protocols prior to fluorescence-activated cell sorting of tumor cells. We conducted RNA-sequencing on three replicates of tumor cell samples from the brain, femurs, adrenals, and ovaries of different mice. Gene expression analysis was done using a combined strategy of prediction analysis for microarrays (PAM) comparing sample sets from each organ, and a supervised rank product analysis comparing brain samples with all the other organ samples (percentage of false positives cut-off < 0.001). Five brain-specific genes were significantly over-expressed in both analyses: *CBS*, *IFI44L*, *MX1*, *XAF1*, and *AC092165.4* – all previously implicated in cancer, several in melanomas and gliomas, but none in brain metastasis. Each gene could also be validated in a rank product-based meta-analysis comparing the brain sample set with each of the other sample sets. At present, we are correlating the brain metastasis gene expression signature with protein expression from immunohistochemistry of human TMAs of brain metastases and mouse brain and peripheral metastases at different developmental stages. These results will be linked, through pathway and drug sensitivity analyses, to clinical data. Furthermore, each gene will be functionally validated *in vivo* by screening a shRNA library targeting all candidate genes.

CB-069. EXPRESSION OF NDRG2 SENSITIZES GLIOBLASTOMA CELLS TO TEMOZOLOMIDE VIA Akt INACTIVATION

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The N-Myc downstream-regulated gene 2 (NDRG2) has been identified as a potential tumor suppressor. Its expression was negatively correlated with the pathological grade of gliomas. The relationship between NDRG2 expression and the effect of temozolomide (TMZ) has not been reported. In the present

study, we analyzed the sensitivity to TMZ of *NDRG2*- overexpressed GBM cell line U-87 MG (U-87 MG/NDRG2) and validated the correlation between the expression of NDRG2 and clinical outcome in 76 GBM patients. WST-1 viability assay of U-87 MG/NDRG2 cells showed that the overexpression of NDRG2 increased their sensitivity to TMZ ($p < 0.001$) and FACS-based cell cycle analysis indicated that TMZ induced significant accumulation in the G2/M phase ($p = 0.039$) followed by senescence and apoptosis (senescence-associated β-galactosidase assay: $p < 0.001$, annexin V binding assay: $p < 0.001$). Akt activation was suppressed in TMZ-treated U-87 MG/NDRG2 cells. Our findings suggest that the overexpression of NDRG2 inactivates Akt and leads to senescence and apoptosis. In GBM patients with unmethylated MGMT, the expression of NDRG2 was statistically correlated with a good prognosis ($p = 0.041$). In conclusion, NDRG2 is a novel therapeutic target in GBM patients in order to sensitize to TMZ.

CB-070. MicroRNA-183 UPREGULATES HIF-1α BY TARGETING ISOCITRATE DEHYDROGENASE 2 (IDH2) IN GLIOMA CELLS

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INTRODUCTION: Our previous study revealed extensive modulation of a set of microRNAs (miRs) in malignant glioma. In that study, miR microarray analysis demonstrated the upregulation of microRNA-183 (miR-183) in glioblastomas. Therefore, we examined the expression levels of miR-183 in various types of gliomas and the association of miR-183 with isocitrate dehydrogenase 2 (IDH2), which has complementary sequences of miR-183 in the 3′-untranslated region (3'UTR). MATERIALS AND METHODS: We searched for the target of miR-183 using the ''miRanda'', ''TargetScan'', ''PicTar'' website, and found that miR-183 contains complementary sequences to the 3'UTR of IDH2 mRNA. We transfected glioma cell lines with miR-183 mimic RNA and analyzed the expression levels of IDH2 and HIF-1α using real-time RT-PCR and western blotting. We also determined the association between miR-183 and vascular endothelial growth factor (VEGF) or glucose transporter 1 (GLUT1), which are downstream molecules of HIF-1α. Furthermore, we generated a luciferase reporter plasmid containing the wild-type or mutant IDH2 3'UTR, and performed luciferase assays by co-transfecting the miR-183 and plasmid into the glioma cells. RESULTS: We demonstrated that miR-183 is upregulated in the majority of high-grade gliomas and glioma cell lines compared with peripheral, non-tumorous brain tissues by real-time PCR analysis. The mRNA and protein expression levels of IDH2 are downregulated via the overexpression of miR-183 mimic RNA in glioma cells. We also verified that miR-183 directly affects the level of IDH2 mRNA in glioma cells using luciferase assays. Further, we showed that expression levels of HIF-1α, VEGF, and GLUT1 are upregulated in glioma cells following transfection with miR-183 mimic RNA by real-time RT-PCR. CONCLUSION: Upregulation of miR-183 in malignant glioma induces HIF-1α expression by targeting IDH2 and may play a role in glioma metabolism.

CB-071. INTEGRATIVE miRNA-mRNA GENOMIC ANALYSIS IDENTIFIES DYSREGULATED miRNAs IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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Epigenetic dysregulation has been implicated in many cancers. Recently, a specific histone mutation was identified in ~80% of DIPG tumors, suggesting a critical role of epigenetic dysregulation in DIPG pathogenesis. MicroRNAs (miRNA) are another important component of the epigenetic regulatory system. Aberrant expression of microRNAs has been shown to play important roles in the oncogenesis of many cancers. To understand the role of miRNAs in DIPG pathogenesis, we performed an integrative miRNA-mRNA genomic analysis of post-mortem DIPG tumors in comparison to normal brain tissues from the same subjects together with an analysis of miRNA-mRNA profiles from DIPG tumorsphere cell lines, normal neural stem cell lines and differentiated DIPG cell lines. Differentially expressed miRNA analysis revealed a group of 25 upregulated and 19 downregulated miRNAs as significantly altered in DIPG. Of the upregulated miRNAs, most are known oncomirs (miR-183 ~ 96 ~ 182 and miR-17-92 clusters). Conversely, many of the down-regulated miRNAs were previously reported as tumor suppressors (miR-29b, miR-124 and miR-145). Interestingly, 11 of the 19 down-regulated miRNAs were located on chromosome 14, which has been found frequently lost in DIPG tumors. Integration of the differentially expressed miRNAs with mRNA profiles predicted enrichment of functional/biological pathways regulated by aberrantly expressed miRNAs, which were under further investigation in DIPG tumorsphere cell lines in vitro. In conclusion, our comprehensive integration of miRNA and mRNA profiling has elucidated miRNA-mediated mechanisms of DIPG pathogenesis and uncovered novel potential therapeutic targets.

CB-072. MUTATIONS OF CD79B ARE COMMON EVENT IN PRIMARY CENTRAL NERVOUS SYSTEM B-CELL LYMPHOMA PATIENTS AND ARE ASSOCIATED WITH UNFAVORABLE PROGNOSIS

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PURPOSE: Constitutive activation of the nuclear factor-kappa B (NF-κB), a transcription factor that regulates cell proliferation, differentiation, and apoptosis, is crucial for cell survival of systemic activated B-cell like diffuse large B-cell lymphoma (ABC-DLBCL) as well as primary central nervous system lymohoma (PCNSL). CD79B mutations occur in a small subset of ABC-DLBCL and trigger chronic B-cell receptor signalling, resulting in NF-kB pathway activation. In this study, we set out to examine the incidence and prognostic impact of CD79B mutation in PCNSL patients. MATERIALS AND METHODS: 61 immunocompetent PCNSL patients with tissue samples available for analysis were included in the study. Exon 5 of CD79B, which contains the sequence encoding the first tyrosine (Y196) of the ITAM domain, was amplified and sequenced. RESULTS: CD79B mutations were identified in 26.2% (16/61) of the cases, confirming the somatic origin or the mutations. Of these, missense mutations affecting the first ITAM tyrosine (Y196) were observed in 87.5% (14/16). The PFS in patients with CD79B-mutated tumors was significantly shorter than those with wild-type tumors ($P = 0.049$, Wilcoxon test). Patients with CD79B wild-type tumors showed a tendency to have longer overall survival, however the difference did not reach statistical significance ($P = 0.13$, Wilcoxon test). CONCLUSIONS: Mutations of CD79B frequently occur and predominantly target the ITAM domain in PCNSL. We propose that the CD79B ITAM domain status is a clinically applicable candidate prognostic marker for PCNSL patients.

CB-073. ELUCIDATING THE MECHANISM OF RECEPTOR TYROSINE KINASE INHIBITION BY SOLUBLE LRIG1 IN GLIOBLASTOMA

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In Glioblastomas (GBM) aberrant receptor tyrosine kinase signaling is found in up to 90% of all cases. A majority of GBMs display gene amplification and overexpression of epidermal growth factor receptor (EGFR) and co-express a mutant form of the receptor, often displaying a truncation in the extracellular domain. The most prominent of the truncated receptors is EGFRvIII which is constitutively active and therefore highly oncogenic. We showed recently that the soluble, extracellular domain of LRIG1, a tumor suppressor and stem cell regulator, strongly inhibits glioma growth in vitro and in vivo in patient-derived GBM xenografts. Local delivery of soluble LRIG1 in pre-established tumors significantly increased survival of tumor bearing mice. LRIG1 belongs to the family of Leucine-rich Repeats and ImmunoGlobulin-like domain proteins and is a negative regulator of EGFR signaling by inducing receptor degradation. Interestingly we found that the tumor growth inhibiting effect of secreted LRIG1 is independent of EGFR expression level and was effective on wildtype and mutant EGFR expressing tumors. Moreover the phosphorylation of EGFR was not affected by exposure to soluble LRIG1. However we observed reduced phosphorylation of its downstream effector MAPK, while AKT signaling was not altered. In order to unravel the mechanism of action of soluble LRIG1 we currently analyze the signaling pathways involved in the response and determined the minimal part of soluble LRIG1 responsible for the tumor growth inhibitory effect in GBM. Using co-immunoprecipitation and gene expression studies, we further address if the effect of secreted LRIG involves the regulation of other receptor tyrosine kinases and its interacting partners in glioma cells. The results from our ongoing studies addressing the mechanism

by which soluble LRIG1 inhibits EGFR and EGFRvIII driven glioma growth will be presented.

CB-074. EXPRESSION OF HUMAN ARYL HYDROCARBON RECEPTOR AND ARNT, ITS NUCLEAR TRANSLOCATOR, IS UPREGULATED AFTER CHEMOTHERAPY IN RECURRENT GLIOBLASTOMA MULTIFORME

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The dioxin/aryl hydrocarbon receptor (AhR) is a transcription factor, which has been attributed a role in human cancerogenesis, cell cycle progression and transforming growth factor-b (TGF-b) signaling. The endogenous tumor-promoting ligand of the human AhR kynurenine was discovered recently. It is constitutively generated by tumor cells via tryptophan-2,3-dioxygenase (TDO), a neuron-derived tryptophan-degrading enzyme. The TDO-AhR pathway is active in human brain tumours and is associated with poor survival. We used real-time RT-PCR to quantify the expression of AhR, TDO, the AhR nuclear translocator (ARNT), and the repressor of AhR (AHRR). We examined a set of 70 human glioma samples representing the differences between the three degrees of malignancy (WHO grade II-IV), primary and recurrent gliomas, pure and mixed astrocytic tumors, and with and without radio- and/or chemotherapy. We also studied whether the expression profile changes as the same tumor progresses in 20 individual patients. Both, the expression of AhR and ARNT were significantly increased after chemotherapy (CTx). The AhR was upregulated from 0.69 before CTx to 3.49 after CTx in secondary GBM and from 0.76 to 1.51 in primary GBM respectively ($p = 0.003$). The mRNA expression of ARNT was increased from 1.37 before CTx to 2.7 after CTx in secondary- and from 0.86 to 1.68 in primary GBM ($p = 0.009$). In contrast, TDO expression was downregulated during temozolomide CTx in GBM from 0.77 to 0.35 ($p = 0.03$). Moreover, ARNT was significantly upregulated in grade II and grade III astrocytomas compared to peritumoral tissue ($p < 0.0001$). TDO expression was significantly higher in GBMs compared to lower grade astrocytomas ($p = 0.001$). AhR and ARNT were significantly upregulated after chemotherapy in glioblastoma. In contrast, TDO was significantly downregulated. These results provide evidence for an important pathophysiological role of the AhR with profound implications for cancer and immune biology and could have broad implications for potential targeted therapies.

CB-075. HETEROGENEITY OF HUMAN GLIOMAS: GLUTATHIONE-S-TRANSFERASE GENE EXPRESSION PROFILE DURING DISEASE PROGRESSION

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The glutathione S-transferase pi gene (GSTP1) is a polymorphic gene encoding active, functionally different GSTP1 variant proteins that are thought to function in xenobiotic metabolism and might play a role in susceptibility to cancer, and other diseases. This placental form of GST is the predominant form in human brain tissue and an important drug-detoxifying enzyme. Our hypothesis was a correlation of this protein to the grade of malignancy and chemoresistance in glioma. Therefore, we set out to measure the GSTP1 expression at the mRNA and protein level in order to elucidate the potential role of GSTP1 expression as a biomarker for disease progression and predictor of chemotherapeutic effect in human glioma. We quantified the expression of the drug-metabolizing gene GSTP1 using real-time RT-PCR. More than 70 astrocytic tumor samples were used including all three degrees of malignancy (grade II-IV), primary and recurrent gliomas, pure and mixed astrocytic tumors, with and without chemotherapy. Moreover, we analyzed longitudinally tumor recurrence and disease progression in individual patients ($n = 13$). In addition, protein expression and localization was evaluated by Western Blotting and immunohistochemistry. There were no changes in GSTP1 mRNA expression, determined by quantitative RT-PCR, between diffuse astrocytomas (1.80 ± 0.85), anaplastic astrocytomas (2.53 ± 2.13), secondary GBM without- (2.48 ± 1.02) and with chemotherapy (2.57 ± 1.40), primary GBM (1.95 ± 1.14) and recurrent primary GBM after Stupp protocol (3.13 ± 1.70). The latter difference could indicate a trend towards an upregulation of GSTP1 after radiochemotherapy in primary GBM, however not a significant one ($p = 0.08$). The expression of GSTP was highly heterogeneous within the surgical specimens. No significant differences in gene expression were detected between primary or recurrent gliomas, suggesting that glioma chemoresistance either intrinsic or might be multifactorial and GSTP1 independent.

CB-076. THE ROLE OF TWIST1 IN GLIOMA FORMATION AND PROGRESSION

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Diffusely invasive growth – a characteristic feature of glioblastoma (GBM) – poses a major challenge to current treatment paradigms and is responsible for the dismal prognosis associated with this condition. Efforts in our lab to elucidate regulatory networks responsible for GBM invasiveness led to the identification of Twist1 as an important mediator of mesenchymal change and, accordingly, of enhanced potential for tumor cell adhesion, migration, and invasion. In human GBM stem-like cells, we have further shown that inhibition of TWIST1 mitigates invasion in vitro and often dramatically inhibits tumor growth in vivo. Together with the observation that TWIST1 is upregulated in grade IV gliomas, this data suggests an important role for TWIST1 in glioma malignancy. However, a fundamental unanswered question is whether TWIST1 is functionally important for tumor formation, tumor progression, or both. To facilitate further study of the role of Twist1 in glioma malignancy, we developed an experimental model for in vitro and in vivo applications, in which Twist1 can be conditionally deleted following exposure to cre recombinase. Mice homozygous for floxed Twist1 were crossed twice with mice harboring the mTmG transgene. Normal neural progenitor cells (NPCs) from these mice were isolated, characterized, and exposed to cre recombinase with near-complete conversion from red to green fluorescence, consistent with successful cre-mediated recombination. To determine the role of Twist1 in tumor formation, TW fl/fl:mTmG+ NPCs were transformed by forced overexpression of H-RAS V12 and dominant-negative p53 or constitutively active AKT1 – combinations intended to align with proneural versus mesenchymal glioma subtypes. Together with future studies defining the impact of TWIST1 abrogation in established gliomas through activation of conditional cre constructs, these approaches will shed light on the functional relevance of TWIST for glioma initiation and progression, and its clinical relevance as a treatment target in gliomas occurring by different oncogenic paradigms.

CB-077. UNDERSTANDING TGF-BETA-MEDIATED Smad1/5/8 SIGNALING IN GLIOMAS: A PREREQUISITE FOR THE DEVELOPMENT OF TGF-BETA-BASED THERAPEUTIC AGENTS

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Glioblastoma is the most common primary brain tumor in adults and the most aggressive of the gliomas. It is characterized by a disproportionately high morbidity and mortality reflected in one of the worst 5-year survival rates among human cancers. Malignant gliomas have become one of the favored scientific vehicles to explore the cancer stem cell hypothesis. Glioma-initiating cells (GIC) may represent a principle hurdle in glioma treatment since they may survive current treatments and repopulate the tumor. Glioma-derived transforming growth factor (TGF)-beta is considered central to the malignant phenotype of glial tumors. Members of the TGF-beta superfamily such as TGF-beta itself and the bone morphogenetic proteins (BMP) have been shown to be involved in the regulation of GIC. Until now, therapeutic approaches targeting glioma-derived TGF-beta have focused on the inhibition of TGF-beta-induced Smad2/3 phosphorylation. BMP reportedly activate Smad1/5/8 phosphorylation in GIC. Interestingly, in endothelial cells, TGF-beta has been reported to signal through recruitment of both the TGF-beta receptor kinase activin-like kinase (ALK)-1 activating the Smad1/5/8 branch and the TGF-beta receptor kinase ALK-5 activating the Smad2/3 branch. Antagonizing the TGF-beta receptor kinase ALK-5 has been shown to inhibit growth and invasiveness and to enhance the immunogenicity of murine and human glioma cells *in vitro* and *in vivo*. This tempted us to investigate whether TGF-beta signals also via Smad1/5/8 in gliomas. We characterized the differential expression of ALK-1 and ALK-5 in glioma cells, including several standard cell lines and GIC models. We find that TGF-beta indeed induces Smad1/5/8 phosphorylation in glioma cells that is mediated by ALK-1. Ongoing studies explore the differential contribution of both receptor pathways to the biological effects of TGF-beta in gliomas. The definition of the roles of the respective molecules in gliomas may be instrumental in defining appropriate therapeutic targets for the development of TGF-beta-based therapeutic agents for glioblastoma.

CB-078. MODULATING PDGF LIGAND AND RECEPTOR SIGNALING IN GLIOBLASTOMA

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Platelet-derived growth factor receptor A (PDGFRA) signaling is an important driver of oncogenesis in GBM. Amplification of the receptor is common, involving 22-38% of adult and pediatric GBM, and activating mutations in PDGFRA occur. In many cases, however, PDGFRA signaling is thought to be ligand dependent, suggesting the importance of the extracellular milieu. Heparan sulfate proteoglycans (HSPGs) regulate cell signaling by interacting with morphogens, growth factors, extracellular matrix and growth factor receptors. A major determinant of the affinity and specificity of HSPG-ligand interactions is the sulfation status of 6O-sulfate (6OS) on heparan sulfate (HS). We recently identified a novel role for the extracellular sulfatase, SULF2, in regulating PDGFRA signaling in GBM, and we demonstrated that ablation of SULF2 confers prolonged survival in murine glioma. Here we elucidate how SULF2 alters PDGFRA signaling in GBM and the factors associated with SULF2 upregulation that could drive oncogenesis. Consistent with our findings on SULF2 in PDGFRA signaling, we found that SULF2 was significantly upregulated ($p = 0.0253$) in PDGFRA-amplified GBM compared to non-amplified tumors ($n = 40$ amplified, 332 non-amplified). In contrast, SULF2 was downregulated ($p = 0.0006$) in EGFR-amplified GBM ($n = 167$ amplified, 205 non-amplified). As SULF regulates extracellular 6OS sulfation, SULF2 could potentially alter binding of PDGF ligands to HS or their release from sequestration. We found that SULF2 treatment of heparan sulfate did not alter levels of HS-bound PDGFA or PDGFB, suggesting SULF2 does not affect binding of PDGF to HS. Studies addressing modulation of ligand availability by SULF2 and PDGFRA localization will also be presented. Interestingly, SULF2 knockdown or genetic ablation caused decreased expression of PDGFB and PDGFRA while SULF2 overexpression resulted in increased expression of PDGFA, suggesting multiple mechanisms of signaling modulation. Thus, SULF2 may promote oncogenic PDGFRA signaling by influencing both ligand and receptor levels in GBM.

CB-079. REDUNDANT INSULIN RECEPTOR AND IGF-1R SIGNALING IN GLIOBLASTOMA

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Increased cancer risks and poor prognosis has been found in patients with obesity and type 2 diabetes. In particular, Type 2 diabetes is associated with poor prognosis in high-grade glioma. These observations can be attributed to, at least in part, increased levels of insulin, IGF1 and IGF2 in circulation and activation of corresponding signaling pathways in cancer. Aberrant activated IGF1R pathway induces downstream oncogenic signalings, such as MAPK and PI3K/AKT, and consequently promotes proliferation, survival, and therapeutic resistance in a range of human cancers. Loss of IGF1R signaling can be compensated by activation of the closely related insulin receptor (InsR). In this study, we interrogate the functions of the InsR and IGF1R pathways in a range of genetically heterogeneous glioblastoma samples. We identified a large subset of glioblastoma tumors that were highly sensitive to the dual InsR/IGF1R inhibitor, OSI-906. We further demonstrated that the InsR/IGF1R pathway represented the major activator of the PI3K/AKT signaling axis in these tumors. The majority of these tumors expressed both InsR and IGF1R. Knockdown of either InsR or IGF1R significantly impaired proliferation and survival in spheroid cultures and further repressed orthotopic tumor growth. In addition, these tumors exhibited varying sensitivities to stimulation by insulin, IGF1 and IGF2, which have drastically different affinities against the homodimers of InsR, IGF1R and the heterodimer of InsR/IGF1R. Finally, we showed that inhibition of the InsR/IGF1R pathway by small molecule inhibitors resulted in strong induction of InsR and/or IGF1R, suggesting a potent negative feedback that may compromise the efficacy of InsR/IGF1R inhibitors. Taken together, our results characterize a highly complex and redundant signaling network regulated by both InsR and IGF1R that represent important therapeutic targets in glioblastoma. Our studies also suggest that controlling insulin and IGF1 levels in circulation may improve clinical outcomes in glioblastoma patients.

CB-080. EGFR PHOSPHORYLATES TUMOR-DERIVED EGFRvIII, DRIVING STAT3/5 AND PROGRESSION IN GLIOBLASTOMA

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A frequently occurring mutation in primary glioblastoma, EGFRvIII (vIII), deletes ligand binding and signals constitutively. How this EGFR allele causes transformation has been elusive because of potential autocrine, paracrine loops triggered by vIII alone or heterodimers of vIII with EGFR. Our inability to fully elucidate and target this signaling has contributed to failed clinical trials in patients with few options for therapy. Here, we document co-expression of EGFR and vIII in primary human glioblastoma, with co-expression driving transformation in a cell intrinsic manner. We demonstrate a unique downstream pathway of STAT signaling triggered by EGFR-catalyzed phosphorylation of vIII, and tumorigenesis in vitro and in vivo. We show that EGFR promotes unidirectional phosphorylation of vIII, phosphorylating STAT even in the setting of kinase-dead vIII, implicating vIII as a substrate for EGFR. Phosphorylation of STAT3 required an allele of vIII competent for nuclear entry, with a nuclear complex formed between vIII and STAT3. Our findings elucidate signaling interactions between EGFR and vIII, and suggest combinatorial targeting of the EGFR-vIII-STAT axis as a therapeutic approach to vIII-mutant glioblastoma.

CB-081. IDENTIFICATION OF MOLECULAR MECHANISMS OF RESISTANCE TO PI3K PATHWAY INHIBITION TO IMPROVE THERAPEUTIC TARGETING OF GLIOBLASTOMA

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Over 50% of human glioblastomas have PIK3R1/PIK3CA/PTEN mutations. PI3 kinase inhibitors produce a partial response, but complete response is rare. In the preclinical experimental models, about half of the responders who benefit from PI3 kinase treatment eventually develop drug resistance after transient response. We propose that, as a result of selective pressure by PI3 kinase inhibitors, glioblastoma: 1) up regulate compensatory survival signaling pathways that, in some cases, may be 'targetable' with an existing drug or combination of drugs, and 2) utilize complimentary pathways as predominant mechanism of escape from anti-PI3K drugs that can be eliminated by concomitant use of complimentary inhibitors. To test these hypotheses, a large panel of glioma cells and glioma Initiating stem cell (GIC) lines were treated with PI3K inhibitor BKM120 or PI3K/mTOR dual inhibitor BEZ235. A differential response to PI3K pathway inhibitors BKM120, BEZ235 divided GICs into responder and non-responder with an aim to identify the molecular profiles that distinguishes the two groups. Gene expression profiling before and after PI3K inhibitor treatment was analyzed by Affymetrix microarrays to identify molecular targets/signatures contributing to resistance to PI3K inhibitors. We identified top 10 up-regulated candidate genes and validated these genes/proteins by quantitative PCR and western blot analysis. Disruption of candidate genes and signaling pathways by specific siRNAs or inhibitors increases the efficacy of PI3K inhibitors in GIC in cell growth assays. To study the molecular mechanisms and signaling pathways contributing to PI3K inhibitor resistance, protein expression/pathway signatures before and after PI3K inhibitor treatment were analyzed by antibody-based proteomics (RPPA). ERK-β-catenin pathway was identified as pathways activated in the non- responders that may lead to resistance to PI3K inhibition. Therefore, our study suggested that targeting essential drug-resistance-related proteins and signaling pathways might allow successful GIC targeting therapy and improve GBM treatment outcome.

CB-082. ACQUIRED RESISTANCE TO EGFR INHIBITORS IN GLIOBLASTOMA IS ASSOCIATED WITH UROKINASE-TYPE PLASMINOGEN ACTIVATOR-MEDIATED SUPPRESSION OF BIM

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The EGF receptor (EGFR) is the most commonly amplified or mutated gene in glioblastoma (GBM), but therapeutic targeting with small molecule tyrosine kinase inhibitors (TKIs) is limited by inherent and acquired resistance. Moreover, GBM is a highly heterogeneous disease and as a result, mechanisms of resistance may be equally heterogeneous from patient to patient. In an effort to model and characterize mechanisms of resistance to EGFR TKIs in GBM we developed *in vitro* and *in vivo* model systems of acquired resistance to the EGFR TKIs gefitinib and erlotinib using TKI-sensitive *ink4a/arf* $-/-$ astrocytes over-expressing the constitutively active mutant ΔEGFR (or EGFRvIII). Clonal TKI-resistant cell lines were generated by chronic drug exposure in two model systems: cells grown *in vitro* as colonies in 3D culture in soft agar or *in vivo* as xenograft tumors. Characterization of TKI-resistant cell lines revealed that although heterogeneous mechanisms do exist, a common feature was the

inability of resistant cells to efficiently undergo apoptosis compared to TKI-sensitive cells. Furthermore, we observed a failure of TKI-resistant cells to induce expression of adequate levels of the pro-apoptotic protein Bim, indicating that a central apoptotic mechanism was altered in the resistant cells. Expression of Bim or restoration of Bim function using BH3 mimetic compounds re-sensitized resistant cells to TKIs, supporting a direct role for this protein in dictating TKI sensitivity. Activation of the cell-surface signaling receptor uPAR, due to increased expression of its ligand urokinase-type plasminogen activator (uPA), was instrumental in suppressing Bim in TKI-resistant GBM cells. Similar results were also obtained in a second independent model of oncogene addiction-driven GBM EGFR TKI resistance. Our findings implicate the uPA/uPAR system as a novel regulator of Bim expression that mediates TKI-resistance in GBM and suggest targeting this signaling axis as a potential therapeutic target in combatting resistance to EGFR inhibitors.

CB-083. IDENTIFICATION AND CHARACTERIZATION OF A NOVEL microRNA INVOLVED IN GLIOMAGENESIS

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PURPOSE: Oncogenic-miRs, which play crucial roles in tumorigenesis, angiogenesis, invasion and apoptosis, have been demonstrated in various types of tumor including glioma. In the present study, we aimed to identify and characterize a novel miR involved in gliomagenesis. METHODS: Total RNAs were extracted from 11 cell lines. Three were tumorigenic glioma cell lines, seven were human primary glioma cell lines, and one was human neural stem cell (NSC). 1347 miRs expression profiling in these cell lines was comprehensively examined by microarray analysis, and aberrantly (more than or less than 2-fold) expressed miRs were identified. To investigate biological roles of an unknown miR in the identified miRs, cell proliferation, cell invasion, cell migration, in vivo tumorigenesis and survival time of the tumor-bearing mice were examined by using glioma cells with knock-downed or overexpressed miRs. In addition, to identify target genes of the new miRs, mRNAs expression profiling was extensively examined by microarray analysis. RESULTS: Seven miRs were aberrantly expressed in all glioma cell lines compared with NSC. Among them, three were up-regulated and four were down-regulated. In these seven miRs, we found only one miR, which was down-regulated, was an unknown molecule. Lentiviral-mediated overexpression of the new miR (miR-X) in glioma cells markedly suppressed cell proliferation, invasion and migration. Furthermore, introduction of miR-X in glioma cells inhibited in vivo tumorigenesis and significantly prolonged survival time of the tumor-bearing mice. Microarray analysis revealed that two genes, which are known to be involved in invasion and migration, were identified as a novel target of miR-X. CONCLUSION: These results suggest that miR-X is a novel molecule to suppress gliomagenesis. Introduction of miR-X in glioma cell or control of the target gene may be a new therapeutic tool for malignant gliomas.

CB-084. LEUKOTRIENE-STIMULATED NESTIN EXPRESSION PROMOTES SONIC HEDGEHOG SIGNAL ACTIVATION AND TUMORIGENESIS OF NEURONAL PROGENITORS

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Medulloblastoma (MB) is the most common malignant brain tumor in children. Its rapid growth and tendency to spread through the nervous system necessitate the use of extreme aggressive therapies. Even with such therapies, a significant proportion of MB patients still succumbs to this disease. Moreover, there is considerable morbidity associated with treatment such as endocrine impairments and cognitive deficits. More effective and less toxic therapeutic strategies to treat this devastating disease are urgently needed. We have previously reported that conditional deletion of Patched1 (an antagonist of sonic hedgehog (Shh) signal) in cerebellar granule neuron precursors (GNPs) causes MB formation in mice, suggesting that aberrant activation of Shh signal is associated with MB tumorigenesis. We recently found MB cells express high levels of the cytoskeletal protein-Nestin. Depletion of Nestin by shRNA significantly inhibits proliferation of MB cells and prevents the growth of MB in mouse, suggesting that Nestin is important for MB progression. We have further revealed that Nestin can stabilize the zinc finger protein Gli3, and subsequently augments Shh pathway activation in MB cells, indicating the important role of Nestin in regulating the output of Shh signal activation. Moreover, Nestin expression in MB cells depends on enhanced synthesis of leukotriene, a lipid derivative. Leukotriene inhibition abolishes Nestin expression in MB cells and simultaneously decreases their proliferation, but has no effect on normal GNP proliferation. These data suggest that inhibition of leukotriene synthesis may represent promising new treatments for human MB. This study sheds light on the essential role of Nestin in Shh signaling activation and the associated medulloblastoma tumorigenesis. Given that leukotriene synthesis is an established drug target, our work paves the road to develop improved approaches to treat medulloblastoma through targeting leukotriene synthesis.

CB-085. BAI1 IS A NOVEL TUMOR SUPPRESSOR IN MEDULLOBLASTOMA

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Brain-specific Angiogenesis Inhibitor 1 (BAI1) is a seven transmembrane G protein-coupled receptor (GPCR) with potent anti-angiogenic and anti-tumorigenic properties. Recent discoveries have shown that the genes encoding BAI family proteins are lost and/or undergo somatic mutations in several cancers, including lung, breast, ovarian and brain, suggesting that BAI proteins might be tumor suppressors. In the present study we provide evidence that BAI1 expression is substantially downregulated in patient-derived samples of medulloblastoma, the most malignant brain tumor in children. Our molecular analyses provide evidence that the gene is epigenetically silenced and we identified several molecular mediators involved in its downregulation. We demonstrate that BAI1 expression can be reactivated by RNA interference against EZH2, a histone methylatransferase overexpressed in MB and MBD2, a DNA methyltransferase. Small molecule epigenetic modulators were also able to reactive gene expression. As an independent means to study the role of BAI1 in tumor suppression we generated Bai1 knockout mice and crossed them with MB-prone transgenic mice. Deletion of Bai1 in mice induced aberrant proliferation of granule neuron precursors and accelerated tumorigenesis in mouse models. Taken together, our novel findings provide insight into the neurobiological mechanisms underlying cerebellar development and its susceptibility to neoplastic transformation.

CB-086. TEMOZOLOMIDE-TRIGGERED ACTIVATION OF THE NA^+-K^+-$2CL^-$ COTRANSPORTER NKCC1 VIA THE WNK1/OSR1 KINASE PATHWAY IN REGULATION OF GLIOMA MIGRATION

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The bumetanide-sensitive Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1) maintains cell volume homeostasis by regulating intracellular K^+ and Cl^- content. NKCC1 expression is associated with high-grade gliomas and increased NKCC1 activity was shown to promote glioma cell migration. The mechanisms underlying enhanced NKCC1 activity, and whether NKCC1 activity is modulated by the chemotherapeutic agent, temozolomide (TMZ), are unknown. In this study, we show that compared to control human neural stem cells and astrocytes, primary glioma cells (GCs) exhibit increased phosphorylation of NKCC1 as well as two upstream regulatory kinases, With-No-Lysine kinases 1 (WNK1) and oxidative stress-responsive kinase-1 (OSR1). siRNA-mediated silencing of WNK1 or OSR1 reduces the intracellular K^+ and Cl^- content of GCs and abolishes NKCC1-mediated regulatory volume increase. Surprisingly, TMZ causes robust activation of the WNK1/OSR1/NKCC1 signaling pathway along with increased GC migration. Pharmacological inhibition of NKCC1 with bumetanide or siRNA knockdown of WNK1 or OSR1 significantly decreases basal GC migration even after TMZ treatment. Together, these results show that glioma cells have higher basal levels of WNK1/OSR1/NKCC1 activity that may be further enhanced by TMZ treatment, and contribute to glioma migration. Therefore, current TMZ therapy may be enhanced by concomitant inhibition of the WNK1/OSR1/NKCC1 signal pathway as a new treatment strategy for inhibiting high-grade glioma cell migration and improving survival.